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Original article

Blood parameters in pediatric myelin oligodendrocyte glycoprotein antibody-associated disorders

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ABSTRACT

Background and objectives: Patients with myelin oligodendrocyte glycoprotein antibody-associated disorders (MOGAD) clinically present e.g. with acute disseminated encephalomyelitis (ADEM), optic neuritis (ON), transverse myelitis (TM) or aquaporin-4-IgG (AQP4-IgG) negative neuromyelitis optica spectrum disorders (NMOSD)-like phenotypes. We aimed to analyze and compare blood parameters in children with MOGAD, AQP4-IgG-positive NMOSD (hence NMOSD), multiple sclerosis (MS) and healthy controls (HC).
Methods: We evaluated differences in complete blood counts (CBC), neutrophil-to-lymphocyte ratio (NLR), platelet-to-lymphocyte ratio (PLR), monocyte-to-lymphocyte ratio (MLR) and C-reactive protein (CRP) between these four groups and within the groups between clinical attack, acute treatment and remission.
Results: Our cohort consisted of 174 children and adolescents with a total of 550 timepoints: 66 patients had MOGAD (202 timepoints), 11 NMOSD (76 timepoints), 58 MS (219 timepoints) and 39 were HC (53 timepoints). At clinical attack, leukocyte counts were elevated in MOGAD compared to remission ($p < 0.001$) and compared to all other groups ($p < 0.001$). NLR was high in MOGAD and NMOSD, and PLR was high in NMOSD, however, after correction for multiple testing these findings did not remain significant. While glucocorticoids caused an increase of leukocyte counts and NLR in NMOSD and MS, these values remained stable during acute treatment in MOGAD. In remission, NLR normalized in MOGAD, while it stayed high in NMOSD. PLR increased in NMOSD and was significantly higher compared to all other groups.
Discussion: Some blood parameters, mainly leukocyte and differential counts, might help clinicians to evaluate disease activity, differentiate relapses from pseudo-relapses and even distinguish between different disease entities.

1. Introduction

Myelin oligodendrocyte glycoprotein-antibody-associated disorders (MOGAD) are acquired demyelinating syndromes of the CNS characterized by a mono- or multiphasic disease course [1,2]. Improved live cell-based assays have detected antibodies to MOG (MOG-IgG) in an expanding spectrum of demyelinating syndromes including acute disseminated encephalomyelitis (ADEM), optic neuritis (ON), transverse myelitis (TM), encephalitis, and neuromyelitis optica spectrum

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Glossary

ADEM	acute disseminated encephalomyelitis
ADEMON	acute disseminated encephalomyelitis followed by optic neuritis
AQP4	aquaporin-4
CBC	complete blood count
CRP	C-reactive protein
IgG	immunoglobulin G
IVIG	intravenous immunoglobulins
MDEM	multiphasic disseminated encephalomyelitis
MLR	monocyte-to-lymphocyte ratio
MOG	myelin oligodendrocyte glycoprotein
MOGAD	MOG-IgG-associated disorders
MS	multiple sclerosis
NLR	neutrophil-to-lymphocyte ratio
NMOSD	neuromyelitis optica spectrum disorders
ON	optic neuritis
PLEX	plasma exchange
PLR	platelet-to-lymphocyte ratio
TM	transverse myelitis

disorders (NMOSD)-like phenotypes [3–5]. A subgroup of patients experiences a relapsing disease course with phenotypes such as multiphasic disseminated encephalomyelitis (MDEM), recurrent ON, ADEM followed by ON (ADEMON), or NMOSD-like phenotypes [6]. The presenting clinical phenotype of MOG-IgG-positive patients strongly depends on the patient's age at onset, with brain involvement seen more commonly in young children and opticospinal phenotypes occurring more often in older children and adults [7]. In adults and older children, a slight predominance towards women and girls can be observed while in younger children both sexes are affected equally [8].

While clinical, radiological, and cerebrospinal fluid features in children with MOGAD have been investigated, less is known about laboratory markers in pediatric MOGAD. However, currently used agents for acute and maintenance therapy mainly affect blood parameters (e.g. leukocytes, lymphocytes) indicating their importance in demyelinating diseases [9–11]. Furthermore, blood parameters and blood cell ratios such as leukocytes, platelets, differential counts, neutrophil-to-lymphocyte ratio (NLR), platelet-to-lymphocyte ratio (PLR), monocyte-to-lymphocyte ratio (MLR) and C-reactive protein (CRP), have been reported as markers for disease activity and progression in various autoimmune [12–15] and neurological diseases [16–21]. One study looked at NLR in a cohort of 62 children with different demyelinating diseases (MOGAD, aquaporin-4-IgG (AQP4-IgG) positive NMOSD, MS). In this study, NLR was high at time of clinical attack and during remission in AQP4-IgG-positive NMOSD (hence NMOSD) and higher only at time of clinical attack but not at time of remission in MOGAD and not different to that of healthy controls (HC) neither during relapse nor remission in MS. This indicates temporal correlation of NLR with disease activity in pediatric MOGAD [22]. Another recent study evaluated leukocyte levels and differential counts in adult patients with NMOSD (n = 13) and MOGAD (n = 26) at time of acute attack and compared the profiles with those in HC and patients with MS. This study found patients with MOGAD had significantly higher leukocyte levels, neutrophil and monocyte counts, MLR and NLR compared to HC. Patients with NMOSD had significantly higher neutrophil counts, monocyte counts, MLR and NLR and lower eosinophil and basophil counts compared to HC. While no significant differences in leukocyte levels and differential counts could be found among MOGAD, NMOSD, and MS, the differential counts in MOGAD and MS appeared to differ from those in NMOSD [23].

Despite their easy availability and low costs, blood parameters have not been used systemically as a diagnostic tool in pediatric MOGAD. The aim of this study is to analyze and compare complete blood counts (CBC) and blood cell ratios in children with MOGAD, NMOSD, MS and HC, possibly helping clinicians to evaluate disease activity, differentiate relapses from pseudo-relapses and even distinguish between different disease entities.

2. Methods

2.1. Patients

Between 2009 and 2023 1235 children with a suspected acquired demyelinating syndrome were sent to our attention from 60 different mainly European medical centers for testing of serum MOG-IgG and AQP4-IgG and included in an ongoing prospective study (i.e. the BIOMARKER cohort). From this cohort, for the purpose of this study, 135 patients were selected who met the following inclusion criteria.

- 18 years and younger at time of diagnosis
- Full dataset (age, sex, MOG- and AQP4-IgG status, diagnosis)
- Assignable to one of three clinical entities: MOGAD, AQP4-IgG-positive NMOSD, MS
- Record of at least one blood sample: CBC, blood cell ratios, CRP
- Timepoint of blood samples assignable to one of three timepoints: (I) clinical attack before treatment initiation, (II) during acute treatment (with high dose methylprednisolone or any other form of acute treatment mostly other glucocorticoids, intravenous immunoglobulins (IVIG), plasma exchange (PLEX) or combinations of these treatments), (III) in remission more than 30 days after symptom onset (patients had to be asymptomatic or have residual symptoms not associated with disease activity, patients could not receive acute treatment but take other medications including maintenance therapy) 39 children were assigned to the healthy control (HC) group. HC had to be afebrile, free of infection and either healthy or under non-immunomodulating treatment for a non-inflammatory disease. Thus, 174 children in total were included in this study.

2.2. Antibody assays

Serum samples were analyzed for the presence of MOG- and AQP4-IgG by live cell-based immunofluorescence assays using human embryonic kidney (HEK293A) cells transfected with full-length MOG and AQP4 (isoform M23) as previously described [24,25]. Titers above 1:160 were classified as MOG-IgG positive. Any AQP4-IgG titers were seen as positive.

2.3. Analyzed parameters

Our study focused on the analysis of blood parameters. These included CBC with hemoglobin, hematocrit, mean corpuscular hemoglobin, mean corpuscular volume, mean corpuscular hemoglobin concentration and the number of platelets. Furthermore, the leukocyte levels were assessed with the absolute and relative numbers of lymphocytes, neutrophils and monocytes and the relative numbers of eosinophils and basophils. Finally, the blood cell ratios NLR, PLR and MLR as well as CRP levels were analyzed.

In addition to the analysis of the patients' blood parameters, their age, sex, MOG- and AQP4-IgG status, diagnosis and medications were evaluated.

2.4. Statistical analysis

Statistical analysis was performed using IBM SPSS. We compared clinical, demographic and serological data using the Kruskal-Wallis test. Bonferroni adjustment was used to correct for multiple testing, Dunn-Bonferroni post hoc test was performed for pairwise comparison. As

median age and sex distribution differed between groups and may be an influential factor in some blood parameters, to avoid data distortion, additional age- and sex-corrected comparisons were performed. Therefore, data was log transformed to meet the assumptions of parametric statistical tests. Age- and sex-corrected comparison between groups was performed for each timepoint using analysis of covariance with subsequent pairwise comparisons. Statistical significance was defined as a two-sided p-value of <0.05.

3. Results

3.1. Patients

174 children and adolescents with a total of 550 timepoints were included in this study: 66 patients had MOGAD (202 timepoints), 11 NMOSD (76 timepoints), 58 MS (219 timepoints) and 39 children were HC (53 timepoints) (see Fig. 1). 47 of 66 patients with MOGAD had monophasic disease courses, and 19 of 66 patients presented with relapsing phenotypes. Median age in MOGAD was 6 years, 14 years in NMOSD and 15 years in MS. Sex distribution was almost equal in MOGAD with slightly more girls than boys (55 %) in our cohort, while in the MS group two thirds of patients were female, and in NMOSD a strong female preponderance (91 %) could be observed (see Table 1).

3.2. Blood parameters

3.2.1. Comparison within disease groups between timepoints

MOG-IgG titer was high in patients with MOGAD at disease onset and during acute treatment and decreased in remission ($p < 0.001$). Leukocyte counts were elevated in MOGAD at clinical attack compared to remission ($p < 0.001$) and remained high but did not further increase under acute treatment. In NMOSD, leukocyte levels were not elevated at clinical attack, significantly increased under acute treatment, and decreased in remission compared to acute treatment. Leukocyte counts were significantly elevated in MS at clinical attack compared to remission, and significantly increased under acute treatment. Relative number of lymphocytes in MOGAD was low at clinical attack and under acute treatment compared to remission ($p = 0.04$) but did not significantly decrease under acute treatment, while in NMOSD and MS lymphocytes were not decreased at clinical attack compared to remission but significantly decreased under acute treatment. Absolute and relative number of neutrophils were high in MOGAD at clinical attack and under acute treatment compared to remission ($p < 0.001$, $p = 0.02$). In NMOSD, absolute and relative number of neutrophils appeared high (median 4.35 G/l, 66.62 %) but were not significantly elevated at clinical attack compared to remission (median 2.89 G/l, 58.99 %), significantly increased under acute treatment (median 9.93 G/l, 74.2 %), and significantly decreased in remission compared to acute treatment but not compared to clinical attack. In MS, absolute number of neutrophils was significantly elevated at clinical attack compared to remission and further increased under acute treatment, while relative number of neutrophils was not significantly increased at clinical attack compared to remission but significantly increased under acute treatment. In our MOGAD cohort, although NLR appeared higher at clinical attack (median 2.27) and under acute treatment (median 2.89) compared to remission (median 1.2) after correction for multiple testing the difference between timepoints did not remain significant. In NMOSD and MS, NLR was not higher at clinical attack compared to remission but significantly increased under acute treatment.

Table 2 shows medians (25th percentile – 75thpercentile) and p-value of timepoint comparisons for the most relevant blood parameters in MOGAD.

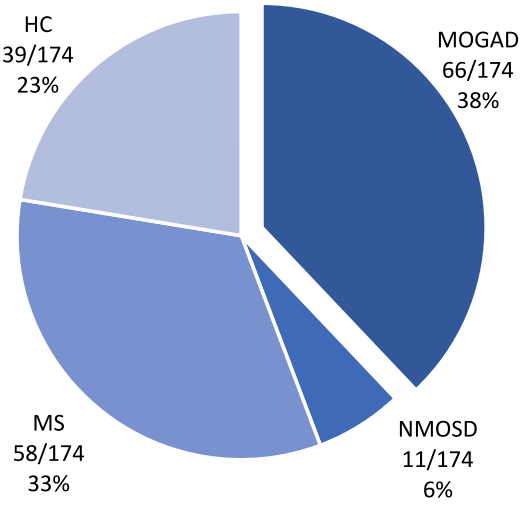

Fig. 1. Pie chart of absolute and relative number of patients per disease group in our cohort: MOGAD 66/174 (38 %), NMOSD 11/174 (6 %), MS 58/174 (33 %), HC 39/174 (23 %).

3.2.2. Comparisons among disease groups at each timepoint

At each individual timepoint, age differed between the three groups with patients with MOGAD being younger than all other patients ($p < 0.001$).

3.2.2.1. Significant differences among disease groups at each timepoint after correction for multiple testing and correction for age and sex. At clinical attack, leukocyte levels, absolute and relative number of neutrophils and NLR were higher in MOGAD compared to MS and relative number of lymphocytes was lower in MOGAD compared to MS. Under acute treatment, no significant differences could be shown. In remission, absolute and relative numbers of lymphocytes were lower in NMOSD than MS and relative number of neutrophils was higher in NMOSD than in MS. PLR was higher in NMOSD compared to the other two groups.

3.2.2.2. More detailed description of the results. At clinical attack, leukocyte levels were higher in MOGAD compared to the two other groups ($p < 0.001$), however, after correction for age and sex the difference between MOGAD (median 11.2 G/l) and NMOSD (median 6.8 G/l) did not remain significant. Under acute treatment, leukocyte counts did not differ significantly among the groups. In remission, leukocyte levels were lower in NMOSD (median 4.8 G/l) compared to both other groups and higher in MOGAD compared to MS (MOGAD: 7.6 G/l; MS: 6.6 G/l) but after correction for age and sex, no significant difference could be shown. Absolute number of lymphocytes was higher at clinical attack in MOGAD compared to NMOSD and under acute treatment in MOGAD compared to NMOSD and MS, however, after age and sex correction these differences did not remain significant. In remission, absolute number of lymphocytes was lower in NMOSD than in MOGAD and MS and higher in MOGAD than in MS ($p < 0.001$) but after correction for age and sex the difference between NMOSD and MOGAD and between MOGAD and MS did not remain significant. Relative number of lymphocytes appeared to be higher at clinical attack in MS compared to MOGAD and NMOSD, but a significant difference could only be observed between MOGAD and MS after correction for age and sex. Under acute treatment and in remission, relative number of lymphocytes appeared to be lower in NMOSD than in MOGAD and MS, a significant difference could only be shown between NMOSD and MS in remission. Absolute number of neutrophils was higher at clinical attack in MOGAD compared to MS ($p < 0.001$) and appeared to be higher in MOGAD than in NMOSD. Under acute treatment and in remission, absolute number of neutrophils was similar between the groups. Relative

Table 1
Number of patients, total timepoints, number of monophasic and multiphasic disease courses in MOGAD, median age and female percentage of patient groups in our cohort.

Clinical phenotype	Number of patients	Total timepoints (event/treatment/remission)	Median age at clinical attack (range) in years	Female (%)
MOGAD	66	202	6 (0–17)	55 (36/66)
MOGAD monophasic	47	120	4 (0–17)	49 (24/47)
MOGAD multiphasic	19	82	9 (1–15)	57 (11/19)
NMOSD	11	76	14 (6–17)	91 (10/11)
MS	58	219	15 (3–18)	66 (39/59)
HC	39	53	13 (2–18)	67 (26/39)
Total	174	550		

Table 2
Medians (25th percentile – 75th percentile) of blood parameters in MOGAD. P-value corrected for multiple testing <0.05 *, <0.01 **, <0.001 ***, ns not significant;[1] significantly different to remission,[2] significantly different to under acute therapy,[3] significantly different to onset/relapse.

MOGAD								
	onset/relapse		acute treatment		remission		p-value	corrected p-value
MOG-IgG titer [1:X]	1280	(640–2560)[1]	1280	(640–2560)[1]	160	(80–1280)[2,3]	<0.001	***
Leukocytes [G/l]	11.2	(7.8–16.2)[1]	11.2	(7.7–15.1)[1]	7.6	(6.1–9.8)[2,3]	<0.001	***
Lymphocytes [%]	27.33	(13.8–40)[1]	23.03	(14–36.5)[1]	39.86	(29.1–46.6)[2,3]	0.002	*
Neutrophils [G/l]	6.67	(3.9–11)[1]	8.25	(5.4–11.8)[1]	3.87	(2.9–5.6)[2,3]	<0.001	***
Neutrophils [%]	64.74	(50–77.7)[1]	70.15	(52–81.9)[1]	47.83	(44.7–63)[2,3]	0.001	*
NLR	2.27	(1.3–5.2)	2.89	(1.37–5.8)	1.2	(1–2)	0.005	ns

number of neutrophils at clinical attack appeared to be higher in NMOSD compared to both other groups and was significantly higher in MOGAD compared to MS after correction for age and sex. Under acute treatment no difference could be observed between the groups and in remission relative number of neutrophils appeared to be higher in NMOSD than in the two other groups with a significant difference between NMOSD and MS after correction for age and sex. At clinical attack, NLR appeared to be higher in MOGAD (median 2.28) and NMOSD (median 2.57) compared to MS (median 1.85), while a significant difference could only be shown between MOGAD and MS after correction for age and sex. Under acute treatment and in remission, NLR appeared to be higher in NMOSD (median 4.94; 2.24) than in MS (median 3.55; 1.61) and MOGAD (median 2.89; 1.2) with the lowest NLR found in MOGAD. PLR appeared to be higher in NMOSD compared to the other two groups at all three timepoints, however, a significant difference could only be shown in remission ($p < 0.001$).

Table 3
Medians of blood parameters at clinical attack, under acute treatment and in remission. P-value corrected for multiple testing and for age and sex <0.05 *, <0.01 **, <0.001 ***, ns not significant;[1] significantly different to MS,[2] significantly different to NMOSD,[3] significantly different to MOGAD. Numbers without apostrophe indicate significant difference only before correction for age and sex, numbers with ‘ with and without age-correction, numbers with ‘‘ only after correction for age and sex.

Clinical attack						
	MOGAD	NMOSD	MS	p-value	Corrected p-value	p-value corrected for age and sex
Leukocytes [G/l]	11.2[1',2]	6.8[3]	7.61[3']	<0.001	***	***
Lymphocytes [G/l]	2.86[2]	1.86[3]	2.32	0.002	*	ns
Lymphocytes [%]	27.33[1]	26.08	30.92[3]	0.068	ns	**
Neutrophils [G/l]	6.73[1]	4.35	4.44[3']	<0.001	***	**
Neutrophils [%]	64.74[1]	66.62	58.55[3]	0.054	ns	*
NLR	2.28[1]	2.57	1.85[3]	0.083	ns	**
PLR	120.56	162.29	117.56	0.028	ns	ns
Acute treatment						
	MOGAD	NMOSD	MS	p-value	Corrected p-value	p-value corrected for age and sex
Leukocytes [G/l]	11.2	12.68	9.75	0.458	ns	ns
Lymphocytes [G/l]	2.61[1,2]	1.5[3]	1.67[3]	0.001	*	ns
Lymphocytes [%]	23.03	16	21.38	0.038	ns	ns
Neutrophils [G/l]	8.25	9.93	7.7	0.623	ns	ns
Neutrophils [%]	70.15	74.2	77.67	0.074	ns	ns
NLR	2.89	4.94	3.55	0.024	ns	ns
PLR	113.26	163.74	146.77	0.069	ns	ns
Remission						
	MOGAD	NMOSD	MS	p-value	Corrected p-value	p-value corrected for age and sex
Leukocytes [G/l]	7.6[1,2]	4.8[1,3]	6.6[2,3]	<0.001	***	ns
Lymphocytes [G/l]	2.76[1,2]	1.4[1,3]	2.15[2,3]	<0.001	***	**
Lymphocytes [%]	39.86	25.84[1‘‘]	33.75[2‘‘]	0.006	ns	*
Neutrophils [G/l]	3.87	2.89	3.48	0.022	ns	ns
Neutrophils [%]	47.83	58.99	54.34	0.035	ns	*
NLR	1.2	2.24[1‘‘]	1.61[2‘‘]	0.011	ns	*
PLR	102.17[2']	180.89[1',3']	117.55[2']	<0.001	***	**

Table 3 shows medians of most relevant blood parameters at clinical attack, under acute treatment and in remission. P-values are depicted corrected for multiple testing and for age and sex.

Figs. 2–4 show boxplots of leukocytes, NLR and PLR in all three disease groups at each timepoint.

3.2.3. Overall comparisons among disease groups and healthy controls

3.2.3.1. Significant differences at overall comparisons among disease groups and healthy controls after correction for multiple testing and correction for age and sex. Leukocyte levels were higher in MOGAD compared to MS and HC and in NMOSD compared to HC. Absolute number of lymphocytes was lower in NMOSD compared to MS. Relative number of was lower in MOGAD and NMOSD compared to MS and HC. Absolute and relative number of neutrophils and NLR were higher in MOGAD and NMOSD compared to MS and HC. PLR was higher in NMOSD than in MS.

3.2.3.2. More detailed description of the results. Leukocyte levels were higher in MOGAD compared to all other groups ($p < 0.001$), however, the difference between MOGAD and NMOSD did not remain significant after correction for age and sex. Absolute number of lymphocytes was highest in MOGAD, while relative number of lymphocytes was highest in HC and the lowest value for both was found in NMOSD. Absolute number of neutrophils was higher in MOGAD compared to all other groups, but a significant difference was only found compared to HC and after correction for age and sex also to MS. HC had the lowest absolute number of neutrophils. Relative number of neutrophils was higher in NMOSD compared to all other groups ($p < 0.001$) but the difference between MOGAD and NMOSD did not remain significant after correction for age and sex. Furthermore, relative number of neutrophils was significantly higher in MOGAD than in HC and after correction for age and sex also in MS. NLR was higher in NMOSD compared to all other groups ($p < 0.001$). After correction for age and sex, the difference between NMOSD (median 3.07) and MOGAD (median 1.9) did not remain significant, but NLR was significantly higher in MOGAD compared to MS (median 1.82) and HC (median 1.67). PLR was higher in NMOSD compared to all other groups ($p < 0.001$), however, after correction for age and sex the difference between NMOSD (median 172.26) and MOGAD (median 108.57) as well as HC (median 117.8) did not remain significant.

Table 4 shows medians of most relevant blood parameters and p-values for overall comparisons corrected for multiple testing and for age and sex.

4. Discussion

In this study of 66 patients with MOGAD, blood parameters at different timepoints such as clinical attack, acute treatment, remission were assessed and compared in children with MOGAD, NMOSD, MS and a group of HC.

Children with MOGAD presented with significantly increased leukocyte levels at clinical attack (median 11.2 G/l) compared to remission (median 7.6 G/l) and compared to the other groups (NMOSD: 6.8 G/l; MS: 7.61 G/l; HC: 7 G/l). This may be due to specific pathogenic mechanisms, more severe and acute inflammation compared to the other clinical phenotypes, younger age, and/or prolonged leukocyte elevation after a preceding infection [26,27]. As patients with MOGAD were significantly younger than patients in all other groups and leukocyte levels are known to be age dependent [28] mildly elevated leukocyte counts as e.g. in remission (MOGAD: 7.6 G/l; NMOSD: 4.8 G/l; MS: 6.6 G/l; HC: 7 G/l) could be explained by age difference. However, while age might play a role, it does not explain the strongly increased leukocyte levels found in MOGAD at clinical attack. Preceding infections are common in case of ADEM but not as common in other MOGAD subtypes [26,29]. Moreover, even if MOGAD occur following an infection, these infections are usually mild not even causing leukocyte elevation. Also, there are usually at least 7–14 days between the infection and the onset of MOGAD, in which a decrease or even normalization of leukocyte levels would be expected [30–32]. While these factors speak against a

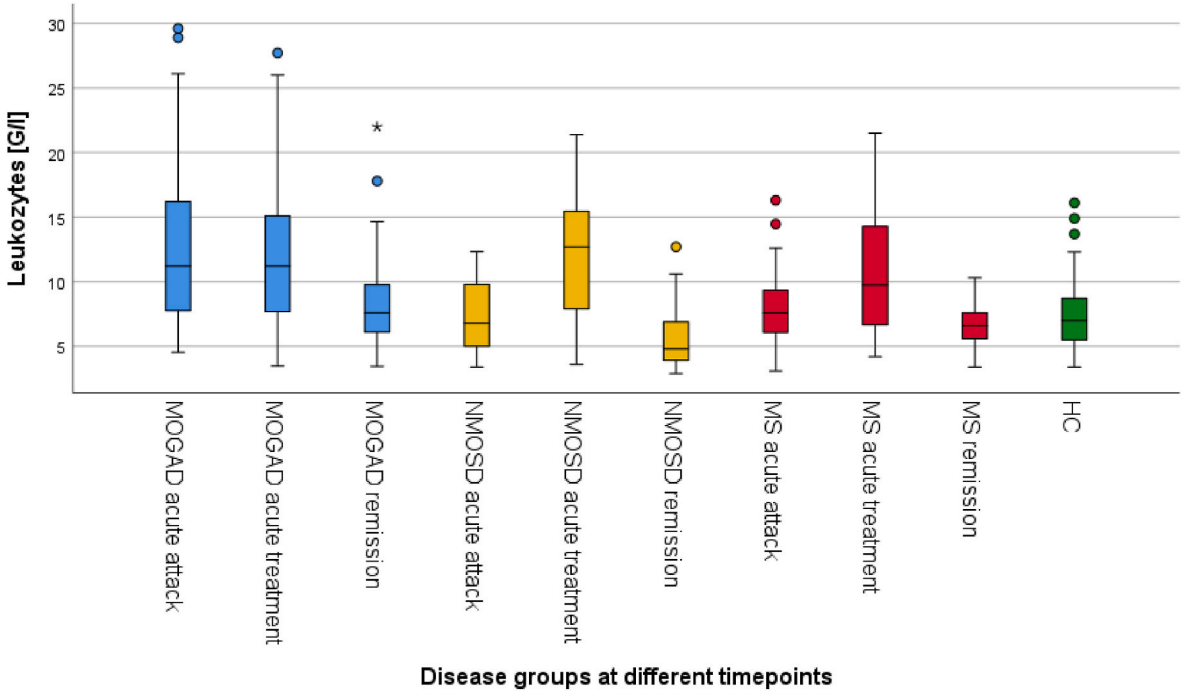

Fig. 2. Boxplot of leukocytes in all three disease groups at acute attack, under acute treatment and in remission. Blue bars representing MOGAD, yellow bars representing NMOSD, red bars representing MS and green bar representing HC. This figure shows leukocyte levels are higher in MOGAD at acute attack and under acute treatment compared to remission and in MOGAD at acute attack compared to NMOSD, MS and HC. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

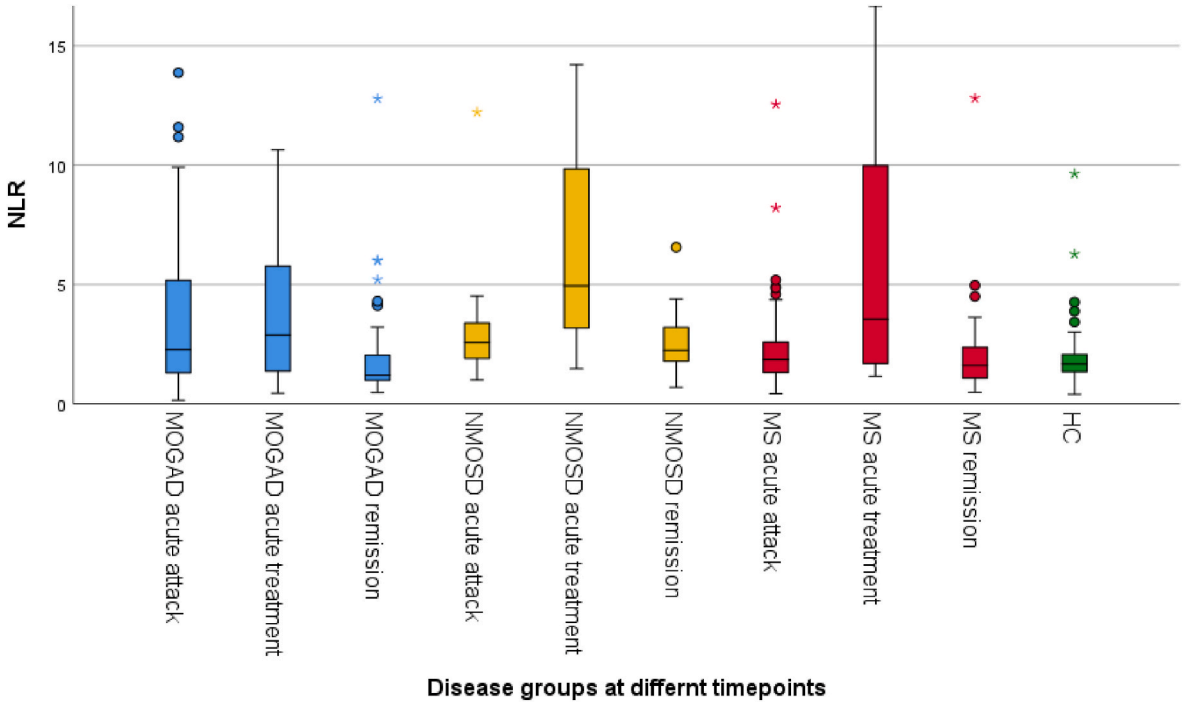

Fig. 3. Boxplot of NLR in all three disease groups at acute attack, under acute treatment and in remission. Blue bars representing MOGAD, yellow bars representing NMOSD, red bars representing MS and green bar representing HC. This figure shows NLR is higher in MOGAD and NMOSD at acute attack compared to MS and HC and stays high in remission in NMOSD compared to MOGAD and MS. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

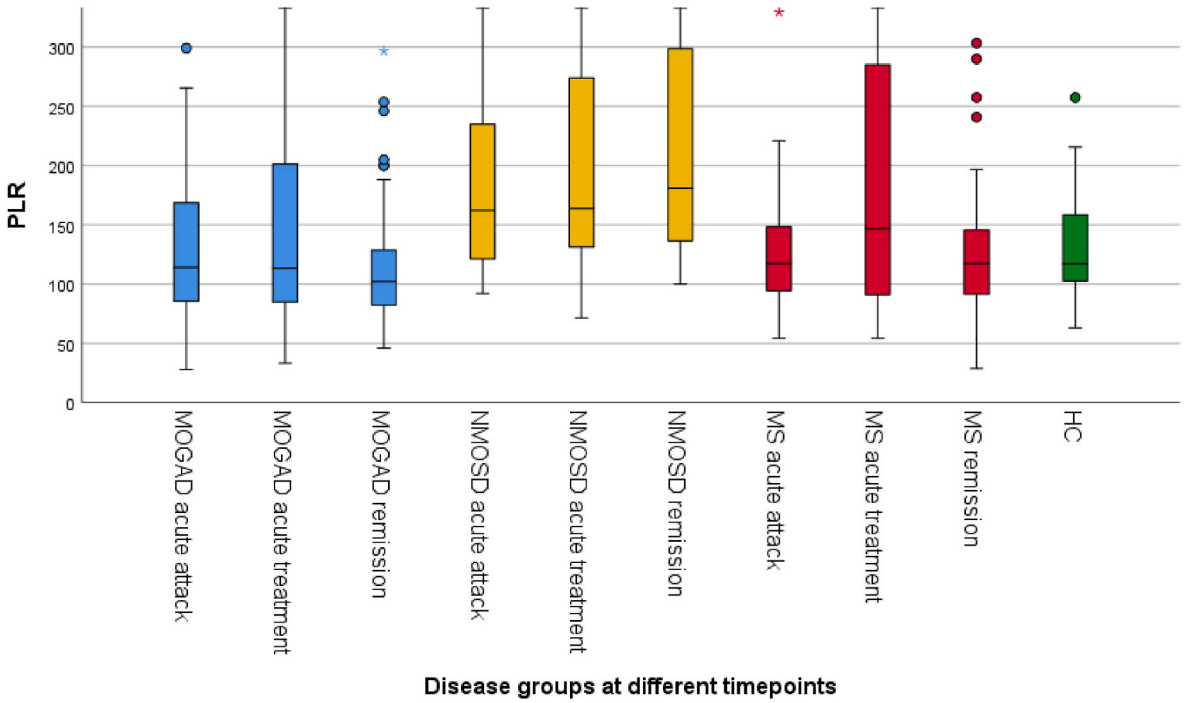

Fig. 4. Boxplot of PLR in all three disease groups at acute attack, under acute treatment and in remission. Blue bars representing MOGAD, yellow bars representing NMOSD, red bars representing MS and green bar representing HC. This figure shows PLR is higher in NMOSD compared to MOGAD, MS and HC. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

leukocyte elevation due to recent infection, all four patients in our cohort who presented with extremely high leukocyte counts (of more than 30 G/l) had MOGAD and three of them had ADEM associated with a reported preceding infection. Therefore, an association between prolonged leukocyte elevation and occurrence of MOGAD cannot be ruled out. Elevated leukocyte levels might help assess disease activity in MOGAD to differentiate relapses from pseudo-relapses. However, it should be considered that elevated leukocyte counts may also be caused

Table 4
Medians of blood parameters in overall comparison. P-value corrected for multiple testing and for age and sex <0.05 *, <0.01 **, <0.001 ***, ns not significant;[1] significantly different to HC,[2] significantly different to MS,[3] significantly different to NMOSD,[4] significantly different to MOGAD. Numbers without apostrophe indicate significant difference only before correction for age and sex, numbers with ‘ with and without age-correction, numbers with ‘‘ only after correction for age and sex.

Overall comparison							
	MOGAD	NMOSD	MS	HC	p-value	Corrected p-value	p-value corrected for age and sex
Age	$6^{1,2,3}$	14^{4}	$15^{1,4}$	$14^{2,4}$	<0.001	***	
Leukocytes [G/l]	$9.82^{1',2',3}$	$7.05^{1'',4}$	$7.1^{4'}$	$7^{3'',4'}$	<0.001	***	***
Lymphocytes [G/l]	$2.8^{2,3}$	$1.5^{1,2',4}$	$2.12^{3',4}$	2.34^{3}	<0.001	***	*
Lymphocytes [%]	$29.82^{1'',2'',3}$	$22.1^{1',2',4}$	$31.54^{3',4''}$	$33.01^{3',4''}$	<0.001	***	***
Neutrophils [G/l]	$6.01^{1',2''}$	$4.4^{1'',2''}$	$4^{1,3'',4''}$	$3.75^{2,3'',4'}$	<0.001	***	***
Neutrophils [%]	$60.81^{1',2'',3}$	$67.61^{1',2',4}$	$57.58^{3',4''}$	$54.89^{3',4'}$	<0.001	***	***
NLR	$1.9^{1'',2'',3}$	$3.07^{1',2',4}$	$1.82^{3',4''}$	$1.67^{3',4''}$	<0.001	***	***
PLR	108.57^{3}	$172.26^{1,2',4}$	$119.9^{3'}$	117.18^{3}	<0.001	***	*

by various other conditions, especially infections [27].

Patients with MOGAD and NMOSD showed elevated neutrophils and NLR and decreased lymphocytes at clinical attack compared to MS and HC. A recent study including 39 adult patients with MOGAD investigated the associations between blood cell ratios and relative number of neutrophils and disease activity. They found higher NLR and relative neutrophils in patients at acute attack compared to remission and suggest relative neutrophils as the simplest and most useful marker to differentiate between attack and remission, showing comparable reliability with NLR in MOGAD [33]. Our results support these findings, showing more significant differences in relative neutrophils compared to NLR in our pediatric cohort. While higher leukocyte levels are partly associated with younger age, neutrophilia is not typical in healthy young children but instead infants normally have higher lymphocyte levels compared to older children and adults [28,34]. However, although viral infections are more likely to cause relative lymphocytosis, some viruses as well as most bacterial infections lead to lymphopenia and neutrophilia, possibly influencing these results [35]. Therefore, these distinct features in MOGAD might again be due to specific pathogenic mechanisms causing neutrophilic inflammation or prolonged blood count changes after a preceding infection [27,36–39].

Leukocyte elevation with increase in neutrophils and decrease in lymphocytes, eosinophils, basophils and monocytes and therefore elevated NLR are known side effects of glucocorticoid therapy [40,41]. While acute treatment with glucocorticoids also caused these typical blood count changes in patients with NMOSD and MS in our cohort, interestingly, the MOGAD group showed no significant difference in leukocyte levels and differential counts compared to clinical attack (before treatment initiation). Therefore, elevated leukocyte counts at clinical attack in combination with lacking blood count changes under glucocorticoids might help differentiate MOGAD from other disease entities and accelerate diagnosis. However, while most of our patients received glucocorticoids as acute treatment, some patients received IVIG, PLEX or combinations of these treatments, potentially influencing these results as different treatments influence blood count parameters differently.

In remission, NLR normalized in MOGAD, while it remained high in NMOSD. PLR was elevated at clinical attack and further increased in remission in NMOSD due to low number of leukocytes and absolute as well as relative lymphocytes. This elevation of NLR and PLR in remission in NMOSD might be due various reasons. First, children with NMOSD almost always have relapsing disease courses. Elevated NLR and PLR in remission might indicate disease activity in asymptomatic periods. Second, AQP4-IgG positive patients often receive long-term treatment. These medications may influence leukocyte levels and differential counts. However, while an influence of long-term treatment on blood parameters is probable, most patients with MS also receive long-term treatment, however, no similar blood count changes could be observed in this group of patients [42–48]. Therefore, pathogenic mechanisms causing permanently low lymphocytes in patients with NMOSD cannot be ruled out and while NLR might be a useful tool to assess disease activity and differentiate relapses from pseudo-relapses in MOGAD, it should not be used to assess disease activity in NMOSD. Instead, it could be hypothesized that NLR and PLR might be useful tools to evaluate the necessity for and/or the effect of long-term treatment in NMOSD.

As we have shown and others previously, MOG-IgG titers decreased in remission [49,50]. Some patients with a relapsing disease course had permanently high MOG-IgG titers, however, most patients showed decrease of MOG-IgG or complete seroreversion within a few months after clinical attack.

Certain parameters (e.g. hemoglobin, hematocrit, mean corpuscular hemoglobin, mean corpuscular volume) showed significantly different values, however, these could be explained by age difference between the groups. For instance, although data was corrected for age and sex, hemoglobin remained significantly higher in MS (median age 15 years, median hemoglobin 138 g/l) compared to MOGAD (median age 6 years, median hemoglobin 124 g/l). Hemoglobin values are known to increase with age [51,52] and there is no known or likely correlation between hemoglobin and demyelinating disorders.

Comparing our results with one study assessing NLR in pediatric MOGAD [22] we could reproduce the observation of NLR elevation at clinical attack in MOGAD (median 2.28) and NMOSD (median 2.57) compared to MS (median 1.85) and HC (median 1.67). Furthermore, we could replicate their results regarding NLR in remission: in MOGAD, NLR decreased from 2.28 to 1.2, while it stayed relatively high, with only a mild decrease from 2.57 to 2.24, in NMOSD. However, in our cohort these differences were not significant. These conflicting results could most likely be explained by a larger number of evaluated parameters in our study and therefore loss of significance through multiple testing. In comparison to another recent study evaluating leukocyte levels and differential counts in adult NMOSD and MOGAD [23] our results coincided regarding significantly higher leukocyte levels, neutrophil count and NLR in MOGAD compared to HC. While they found significantly elevated monocyte count and MLR in MOGAD at clinical attack compared to HC, we could not replicate these findings with even lower monocyte count and MLR in MOGAD (median 5.9 G/l; 0.2) compared to the HC group (median 7.9 G/l; 0.25). Furthermore, adult patients with NMOSD had significantly higher neutrophil and monocyte counts, MLR and NLR and lower eosinophil and basophil counts compared to HC. While pediatric patients with NMOSD also had higher neutrophil counts and NLR and lower eosinophil and basophil counts compared to HC after correction for age and sex no significant difference could be observed. Said study could not show significant differences in leukocyte levels and differential counts among MOGAD, NMOSD, and MS, however, our results show significantly higher leukocyte levels in MOGAD compared to the two other disease groups. Moreover, we found significantly higher neutrophils and NLR and lower lymphocytes in MOGAD compared to MS. Conflicting results may be due to age difference between the two cohorts (median 6 years versus 43 years) and/or

larger cohort size of our study leading to more significance in group comparisons.

In paragraph 3.2.3 we perform overall comparisons among disease groups and HC. In this paragraph we do not differentiate between timepoints within the disease groups. While we feel this comparison adds value in demonstrating general differences among the groups and HC independent of the individual timepoints, these results clearly have to be interpreted with caution as influential factors (including treatments) should be taken into consideration.

In summary, elevated leukocyte counts at clinical attack and missing increase of leukocyte counts under acute treatment might help differentiate MOGAD from other disease entities. High leukocyte levels may also be a useful tool in assessing disease activity in MOGAD and differentiate relapses from pseudo-relapses. Lymphocytes were low, neutrophils and NLR were high in MOGAD and NMOSD, and PLR was high in NMOSD at clinical attack. While these values remained stable during acute treatment in MOGAD, glucocorticoids caused increase of neutrophils and NLR and decrease of lymphocytes in NMOSD. In remission, NLR normalized in MOGAD while it stayed elevated in NMOSD. PLR further increased in NMOSD. Elevated NLR in MOGAD and NMOSD at clinical attack may distinguish them from other disease entities, however, not from each other. Elevated NLR at clinical attack in combination with no further elevation under glucocorticoids may help diagnose or at least consider MOGAD more rapidly. Decrease of NLR in remission may help assess disease activity in MOGAD and differentiate relapses from pseudo-relapses, whereas high NLR and PLR in remission might be associated with long-term treatment, ongoing disease activity in relapsing disease courses or specific pathogenic mechanisms in NMOSD.

While we speculate that in MOGAD certain blood parameters and blood cell ratios could be used as markers for disease activity not only at acute attack but also ongoing disease activity in asymptomatic phases and may be helpful in identifying patients at risk of relapse, our results cannot give a final answer to this question. A comparison of patients with monophasic and multiphasic MOGAD in remission could be useful to assess these parameters as markers for multiphasic disease courses.

Therefore, regardless of our results, more research with pediatric patients with MOGAD will be needed to evaluate blood parameter observations on a larger scale.

4.1. Limitations

Due to the retrospective assessment of the evaluated parameters, one or more parameters are missing in some patients. Furthermore, it must be assumed that certain variables that potentially affect the results may not have been recorded or, as data was collected from multiple centers, may not have been forwarded and therefore were not available, e.g. exact time of acute treatment initiation as well as duration. Although, start and end of treatment were assessed as accurately as possible, it cannot be ruled out that in some cases missing information led to assignment of blood counts to the wrong timepoint.

A further limitation is the age difference between our disease groups, as children with MOGAD are mostly infants while MS is far more common in adolescents. Age is known as influential factor in some blood parameters. Furthermore, sex influences blood parameters especially in older children, and percentages of male and female patients also differed between clinical entities, with 91 % female in NMOSD and only 55 % in MOGAD. Although, to avoid data distortion, values were statistically corrected for age and sex, some of the results are more difficult to interpret due to these differences. Furthermore, while most patients in our cohort received glucocorticoids as acute treatment, some received IVIG, PLEX or combinations of these treatments, likely influencing blood count parameters in different ways.

An additional factor possibly influencing blood parameters in remission may be the immunomodulatory regime. While most patients with NMOSD and MS take some sort of maintenance therapy in remission due to expected multiphasic disease courses, many patients with MOGAD do not need ongoing immunomodulatory treatment in remission as monophasic disease courses are common.

Another limitation that should be mentioned is the difference in group sizes. For instance, while 66 patients in our cohort had MOGAD, only 11 had AQP4-IgG-positive NMOSD. Some seemingly relevant differences were not significant, probably due to small group size. One more limitation, probably influencing our results, is that due to not normally distributed data non-parametric testing had to be used for most calculations and as 20 parameters were assessed p-values had to be corrected for multiple testing (p-value x 20), likely leading to less significant results as well.

5. Conclusion

Some blood parameters, mainly leukocyte and differential counts as well as NLR and PLR, might help clinicians to evaluate disease activity, differentiate relapses from pseudo-relapses **and even distinguish between different disease entities. However,** more research with pediatric patients with MOGAD will be needed to evaluate blood parameter observations on a larger scale.

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Ethics approval

This study was approved by the Ethics Committee of the Medical University of Innsbruck, Austria (Study number AN4095).

Patient consent

All patients and their caregivers gave written informed consent.

Data availability

Any data not published within the article will be made available in anonymized form on request from any qualified investigator.

Author's contribution statements

Alina Peternell and Christian Lechner contributed equally to this work. Kevin Rostásy and Matthias Baumann are shared senior authors. Matthias Baumann is the corresponding author. All authors contributed to study conception, study design and data collection. Data analysis was performed by Alina Peternell, Christian Lechner and Markus Reindl. The first draft of the manuscript was written by Alina Peternell and Christian Lechner, and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Declaration of competing interest

Alina Peternell, Christian Lechner, Mareike Schimmel, Joachim Zobel, Martin Preisel, Eva-Maria Wendel and Astrid Eisenkölbl have nothing to declare. Markus Breu received speaker honoraria from Sanofi Genzyme. Markus Reindl is supported by research grants from the Euroimmun, and Roche, and consulting fees and advisory board from Roche (to institution). Markus Reindl works at the Clinical Department of the Medical University of Innsbruck (Innsbruck, Austria), which offers diagnostic testing for MOG-IgG and other autoantibodies. Matthias Baumann received compensation for advisory boards and speaker honoraria from Novartis, Biogen, and Roche. Kevin Rostásy serves as

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