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Highlights

- PINS appears as myelitis (44%), encephalomyelitis (44%) or encephalitis (12%)
- PINS involve the peripheral nervous system in 41% of cases
- The new set of MS risk alleles does not increase the risk of developing PINS
- MS and PINS have a different etiology, and they need to be treated differently

Impact of multiple sclerosis risk loci in postinfectious neurological syndromes

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ABSTRACT

Background: The genetic component of multiple sclerosis (MS) is now set to 200 autosomal common variants. However, it is unclear how genetic knowledge be clinically used in the differential diagnosis between MS and other inflammatory conditions like adult-onset postinfectious neurological syndromes (PINS). The aim of this study was to investigate whether PINS and MS have a shared genetic background using an updated polygenic risk scores.

Methods: Eighty-eight PINS patients have been consecutively recruited between 1996 and 2016 at Mondino Foundation of Pavia, diagnosed according to clinical, MRI and CSF findings and followed-up for several years. Patients were typed using Illumina array, and genotypes imputed using the 1000 Genomes Project reference panel. A weighted genetic risk score (wGRS) has been calculated based on autosomal MS risk loci derived from large-scale studies, and an HLA genetic burden (HLAGB) was also calculated on loci associated to MS.

Results: PINS occurred as an episode of myelitis in 44% of patients, encephalomyelitis in 44%, and encephalitis in remaining cases, with an involvement of peripheral nervous system in 41% of patients. Mean age of onset was 50.1 years, and female:male ratio was 1.4. Patients were followed-up for a mean of 7.2 years, and at last visit 55% had a low disability grade (mRS 0-1). Disease was monophasic in 67% of patients, relapsing in 18% and chronic-progressive in 15%.

The wGRS of PINS cases was comparable to 370 healthy controls, while significantly lower compared to 907 bout-onset MS (BOMS) cases (wGRS= 20.9 vs 21.2; $p<0.0001$). The difference was even larger for PINS with peripheral nervous system involvement (wGRS=20.6) vs BOMS.

Conclusion: The distinction between MS and PINS is not easy to make in clinical practice. However, our study shows that the new set of MS risk alleles does not confer increased susceptibility to PINS. These data support the importance to discriminate these cases from MS with pathophysiological and therapeutic implications.

Keywords: multiple sclerosis, postinfectious neurological syndrome, acquired demyelinating disease, genetics, weighted genetic risk score.

1. INTRODUCTION

Postinfectious neurological syndromes (PINS) are a spectrum of heterogeneous disorders affecting the central nervous system (CNS), with or without the involvement of peripheral nervous system (PNS), that often follow an infectious event or a vaccination (Brinar and Poser, 2006; Marchioni et al., 2013). Acute disseminated encephalomyelitis (ADEM) is one of the most representative entities of this spectrum. ADEM has been classically defined as an acute, monophasic, and wide demyelination of CNS (Pohl et al., 2016). A PNS involvement, which could not be classified as ADEM, has been reported in adult patients, making it difficult to reach a consensus on diagnostic criteria (Marchioni et al., 2005; Young et al., 2008). A recent classification has been suggested by us in 5 distinct entities based on anatomical site location and the involvement of PNS: myelitis, encephalitis, encephalomyelitis, and, in case of PNS involvement, encephalomyeloradiculoneuritis and myeloradiculoneuritis (Marchioni et al., 2013). Good functional recovery is frequent but not invariable, and a non-negligible proportion of patients experience subsequent relapses or a chronic progressive course (Dale et al., 2000; Koelman et al., 2016; Marchioni et al., 2005).

To date, we do not dispose of biomarkers of diagnosis, outcome or relapse in PINS patients. As a result, immune-modulatory therapies are rarely used, mainly in the late phase of disease course. Moreover, and more importantly, in most cases the diagnosis of PINS is based on the exclusion of other clinical conditions like MS, neuromyelitis optica spectrum disorders (NMOSD) and vasculitis. Similarly to other inflammatory diseases of the CNS, the pathogenesis of PINS is thought to be influenced by both genetic and environmental factors (Waubant et al., 2016). Only few studies have explored the genetic factors predisposing to this condition, and they more frequently addressed pediatric population presenting with “classical” ADEM phenotype (Alves-Leon et al., 2009; Idrissova Zh et al., 2003; Imbesi et al., 2012; Oh et al., 2004). Initial results from these studies suggest that the genetic loci associated with PINS might differ from those associated with MS (Idrissova Zh et al., 2003; Imbesi et al., 2012; Oh et al., 2004).

Given the recent identification from a large-scale international effort (IMSCG, 2019) by the International Multiple Sclerosis Genetic Consortium (IMSGC) of 200 autosomal common variants associated with MS and thanks to the assessment of classical HLA alleles associated with MS in large meta-analysis (Moutsianas et al., 2015), in this paper we sought to: a) describe the clinical and paraclinical features of a cohort of adult PINS patients; b) determine whether PINS and MS have a shared genetic background using polygenic risk scores based on the 200 autosomal MS-risk variants and on classical alleles in the HLA region. Results in PINS patients were thus compared to an historical cohort of bout-onset MS (BOMS) patients and healthy controls (HC).

2. MATERIALS AND METHODS

2.1. Study population

We performed a retrospective research in the Institutional database of IRCCS Mondino Foundation (Pavia, Italy) for all patients diagnosed with encephalomyelitis between 2001 and 2015, using the two ICD-9 codes "323" (encephalitis, myelitis and encephalomyelitis) and "341" (other demyelinating diseases of central nervous system). The clinical records of these patients were critically revised to evaluate study eligibility. Inclusion criteria were: a) acute/subacute onset of neurological signs and symptoms corresponding to encephalitis, myelitis or both; b) inflammatory findings on MRI and/or CSF analysis (pleocytosis and/or protein elevation and/or oligoclonal bands); c) exclusion of alternative conditions including CNS infections and dysimmune conditions of other etiology (e.g., vasculitis, sarcoidosis). Preceding infection (or vaccination) was not considered mandatory for including the patient in the study. Exclusion criteria were: a) meeting McDonald's diagnostic criteria for MS (Thompson et al., 2018a); b) detection of pathogenic antibodies in serum and/or CSF, including neuronal cell-surface, anti-AQP4 and anti-GQ1b antibodies; c) inadequate information on the paraclinical assessments performed to exclude alternative diagnoses.

The local Ethics Committee approved the study, and written consent was obtained from all recruited participants. BOMS and HC subjects were recruited at OSR in agreement with the approval of the local Ethics Committee.

2.2. Genotyping and Imputation

Whole blood sampling (10 ml in EDTA tubes) of enrolled patients were collected in anticoagulant tubes and appropriately identified with unique study number. Genomic DNA was extracted from whole blood at the IRCCS Foundation Mondino Neurological Institute using the FlexiGene DNA kit, QIAGEN GmbH according to manufacturer's instructions and eluted in TE buffer. Quality and quantity controls were performed on extracted DNA: concentration was determined by NanoDrop 8000 Spectrophotometer (Thermo Scientific) and agarose gel at 1% to check for absence of degraded DNA. Genotyping was performed starting from 200 ng of high quality DNA of each sample and genotype calls determined with Illumina HumanOmniExpress-24 BeadChip, following manufacturer's instruction. The Illumina iScan System was used to scan the chip recording high resolution images of the light emitted from the fluorophores. For each sample, normalized bead intensity data was converted to genotypes using the auto-calling algorithm in the Illumina GenomeStudio software.

We applied standard filters for quality control assessment on PINS cohort, at sample and SNP level using PLINK tool (Purcell et al., 2007). More precisely, samples were discarded if they had a call-rate <95%, whereas we removed SNPs with MAF<5%, genotypic call-rate<95% and departing from Hardy-Weinberg equilibrium at $p=10^{-4}$. We thus constituted a cohort of BOMS (n=907) and HC samples (n=370), already available at the Laboratory of Genetics of Neurological Disorders at Ospedale San Raffaele (OSR), and genotyped on different Illumina arrays (OmniExpress-12, OmniExpress-24, Omni2.5M), already quality-controlled with the same set of filters. The two cohorts were merged with PINS cohort on shared markers (n=525,411) for subsequent imputation on 1000 Genomes Phase 3 (ALL reference panel) and HLA imputation. We screened the overall

study cohort (N=1365) for evidence of cryptic relatedness, estimating excess of pairwise identity-by-descent (IBD) with pi_hat<0.25, which measures the proportion of shared IBD alleles. No evidence of duplicated or related samples in IBD analysis was detected.

Imputation was performed on the 1000 Genomes Phase 3 (ALL reference panel) using SHAPEIT2 (Delaneau et al., 2013) for pre-phasing of haplotypes and Minimac3 (Das et al., 2016) for genotype imputation, discarding rare SNPs (MAF<1%) and those with poor or modest quality metrics (Rsq<0.8 as of Minimac3) in post-processing of imputed genotypes. Best-guess genotypes were used in wGRS calculation.

Classical HLA alleles were imputed using SNP2HLA (Jia et al., 2013), based on a reference panel of 5,225 subjects of European ancestry from the Type 1 Diabetes Genetics Consortium, genotyped on 8,926 common SNPs spanning the MHC region. After imputation, we retained alleles with high quality imputation score (r^2>0.8).

2.3. Statistical Analysis

To evaluate the cumulative risk conferred by MS susceptibility loci, a weighted genetic risk score (wGRS) was calculated for each subject as a weighted sum of MS risk alleles (De Jager et al., 2009) for the 200 independently associated autosomal non-MHC loci (IMSCG, 2019), using log-transformed reported odds ratios (OR) as weights. We additionally included in the score low-frequency coding SNPs found associated in the study focused on autosomal exons (IMSGC, 2018). When a SNP was not available on post-QC imputed set, we used a proxy SNP at r^2>0.4, evaluated from EUR panel of 1000 Genomes with LDlink web tool (Machiela and Chanock, 2015), incorporating r^2 value into calculation.

We then computed HLA genetic burden (HLAGB), selecting classical alleles as reported in a previous large meta-analysis (Moutsianas et al., 2015) on the influence of HLA loci in MS risk. We chose 10 out of the 13 loci that were found to be associated with MS according to an additive or dominant model, excluding those showing evidence of interaction. Four were class I (*A*02:01*,

*B*38:01*, *B*44:02*, *B*55:01*) and 6 class II genes (*DRB1*03:01*, *DRB1*08:01*, *DRB1*13:03*, *DRB1*15:01*, *DQB1*03:02* and rs9277565, tagging *DPB1*03:01* allele). We calculated the sum of imputed HLA alleles, weighted with fixed-effect meta-analysed ORs on log scale as reported in the Supplementary Table 1 (Moutsianas et al., 2015).

Our primary analysis compared the wGRS and HLAGB of PINS patients with BOMS and HC. As secondary analyses we investigated wGRS and HLAGB scores in PINS patients with and without PNS involvement, also comparing them with BOMS patients. We further tested the association of the two scores with prognosis at follow-up using the modified Rankin Scale (mRS) dichotomized in two categories (<=1 and >1).

Differences in mean wGRS were evaluated with t-test with Welch's correction of degrees of freedom for unequal variances, whereas Mann-Whitney U test was employed for HLAGB scores. Statistical significance was declared at level $\alpha=0.05$. Since sample size of PINS, BOMS and HC cohorts were substantially unbalanced, we evaluated consistency of results by sub-sampling 1000 times without replacements BOMS and HC cohorts down to PINS cohort's sample size (n=88), in order to estimate significance of wGRS and HLAGB scores in a balanced dataset. Analyses were carried out in GraphPad Prism (v. 5.0) and R (v. 3.4.1) statistical environment (www.R-project.org/). Discriminatory capacity of the two scores was assessed via estimation of the area under the receiver operating curve (AUC) using *ROCR* R package (Sing et al., 2005), after incorporation of the two scores in logistic regression models.

3. RESULTS

3.1. Description of PINS cohort

The steps of patients' selection and reasons for exclusion is summarized in a flow-chart (Figure 1). Out of 230 patients affected by encephalomyelitis according to ICD-9 codes, 8 converted to MS and 17 received alternative diagnosis including NMOSD, encephalitis due to other conditions, CNS

neoplasm and others. Out of 205 patients, 109 were unavailable because lost to follow-up (77) or deceased (32), and additional 8 patients were excluded after genotyping because of alternative diagnosis, reaching a total of 89 patients included in this study. Table 1 summarizes the clinical and paraclinical features of the 88 patients included in the analyses. Fifty-nine patients (68%) had a prodromal infectious event, mainly consisting of isolated fever or flu-like syndrome, and 5% received a vaccination within days or weeks before neurological symptom onset. According to clinical and radiological findings, patients were diagnosed as affected by myelitis, encephalomyelitis or encephalitis, and a PNS involvement was documented in 28 of 68 patients (41%) who were investigated using electroneurography. Of them, half presented as encephalomyeloradicoloneuritis, and the other half as myeloradicoloneuritis. A demyelinating pattern was the most common finding on nerve conduction studies in these patients (57%), axonal in 39% and mixed in 4%.

The most common neurological symptoms included myelopathy (82%), encephalopathy (24%), and brainstem/cerebellar dysfunction (15%). Ten patients (12%) had mono- (6 patients) or bilateral (4 patients) optic neuritis.

All patients but one was investigated by contrast brain and spine MRI. Neuroimaging disclosed inflammatory CNS alterations in 79 cases (91%), and spinal cord was the most affected site (80%). The involvement of basal ganglia (7%) or corpus callosum (3%) was uncommon. Data about contrast enhancement were available only for 78 patients: among them, 59% had contrast-enhancing lesions.

CSF data at disease onset were available for 80 patients. Out of them, 75% had elevated CSF protein levels and/or pleocytosis. Oligoclonal bands (OBs) were found in 38 out of 76 patients tested (50%): 17 presented a mirror pattern (type III), 16 a CSF-restricted pattern (type II) and 5 a mixed pattern (type IV). Half of the patients with CSF-restricted OBs had a second CSF analysis during follow- up, and in 6 cases OBs reduced in number or disappeared.

At the peak of disease severity, 73 patients (83%) showed disability of any grade, defined as a score of mRS>1. Three patients (3%) developed a life-threatening encephalopathy, requiring intensive care. Treatment with high-dose intravenous steroids (methylprednisolone 1 g/day for 5 to 6 consecutive days) was administered to 89% of patients. Of them, 72% had a good response to steroids, defined as a reduction of at least one point on the mRS scale. In 16 patients (18%), intravenous immunoglobulins were administered in the acute phase, due to an unsatisfactory response to steroids. Eleven patients treated with intravenous immunoglobulins had a good response, as defined above. At discharge, the percentage of patients with mRS>1 decreased from 83% to 51%.

The median follow-up duration in our cohort was 78 months (range: 6 – 345 months). Fifty-nine patients (67%) had a monophasic disease. Sixteen patients (18%) experienced clinical relapses during follow-up, defined by an acute/subacute worsening of previous symptoms or onset of new symptoms, associated to the detection of active lesions on MRI. Relapses were more commonly confined to the spinal cord (62% of cases). Thirteen patients (15%) experienced a slow but relentless clinical progression in absence of new MRI lesions. Seven patients with relapsing or progressive disease received azathioprine, cyclophosphamide or rituximab as maintenance therapy, but in most cases these agents failed to prevent progression or relapses. At follow-up, 55% patients had no disability (mRS 0-1), while 31% were severely dependent on daily activities (mRS=4-5).

3.2. Analysis of wGRS and HLAGB scores

As of quality control of genotyped PINS patients (n=89), we excluded one subject with call-rate <95% (Figure 1). The resulting cohort of PINS patients (n=88) was merged with the BOMS (n=907) and HC (n=370) on 525,411 overlapping SNPs, which was the starting set of markers for imputation.

One hundred and seventy four SNPs were present in our imputed dataset, whereas 17 SNPs were neither present nor tagged by proxies at r^2>0.4: with this threshold, we retrieved proxies for 9 SNPs.

As regarding rare variants from Exome Chip study (IMSGC, 2018), we found only one of the seven reported loci (rs35947132). In total, 184 SNPs were selected for the calculation of wGRS, and they are reported, together with proxy variants, in Supplementary Table 1.

As of HLA loci, we imputed the same set of 1,365 merged subjects with SNP2HLA tool, finally obtaining the 10 selected HLA classical alleles. Of these, three were excluded (HLA-A*02:01, HLA-B*38:01, rs9277565) due to modest imputation quality at $r^2<0.8$. Supplementary Table 2 reports the 7 imputed alleles for the 88 PINS patients.

We then investigated the differential distribution of wGRS in the three cohorts, contrasting PINS patients with BOMS patients and HC. The distribution of wGRS fairly approximated a normal distribution, whereas a marked departure from normality was observed in QQ plots for HLAGB (Supplementary Figure 1). The mean wGRS score for PINRF-2011-02347955S, BOMS and HC was 20.86±0.82, 21.23±0.84 and 20.69±0.86 respectively, with a significantly higher value in BOMS cohort compared to PINS ($p<0.0001$), but not between PINS and HC (Figure 2a). These findings were corroborated in the balanced analysis, with 1000 random sub-samplings of 88 subjects from MS and HC datasets, which confirmed a significant difference between PINS and BOMS ($p=0.0021$) and no difference between PINS and HC. When considering contribution of HLA loci, we again observed a similar pattern, with a significantly higher mean HLAGB in BOMS subjects compared to PINS ($p<0.0001$, Mann-Whitney test) and no evidence of difference in mean score between PINS and HC (Figure 2b). The mean HLAGB score for PINS, BOMS and HC was 1.61±0.48, 1.89±0.65 and 1.61±0.49.

We next investigated the discriminative capability of the wGRS score for the two contrasts, performing a ROC analysis. We found that the wGRS was moderately able to distinguish BOMS and PINS patients (AUC=0.63), with a slight increase in discrimination for HLAGB (AUC=0.66); no discrimination power was observed when comparing PINS and HC, either with wGRS (AUC=0.51) or HLAGB (AUC=0.56) (Supplementary Figure 2a-b).

As regarding secondary analyses, we detected a higher wGRSRF-2011-02347955 mean score ($p=0.019$, Figure 3) in PINS patients without PNS involvement (n=60) as compared to those with PNS involvement (n=28), whereas this difference was not detected with HLAGB score. We then compared these PINS subgroups with BOMS subjects, observing a marked separation in terms of wGRS between BOMS and PINS with positive PNS involvement ($p<0.0001$) as compared to PINS with negative PNS involvement ($p=0.038$) (Figure 3). Finally, in the PINS cohort, we did not detect statistically significant differences between the two dichotomized groups for mRS having a different long-term prognosis (Figure 4).

4. DISCUSSION

The diagnosis of PINS in adults can be troubling due to the lack of consensus on diagnostic criteria and the heterogeneous modality of clinical presentation. Furthermore, there is still some debate on the existence of PINS as a separate clinical entity. Main characteristics of these conditions are: a) an acute/subacute CNS or mixed CNS+PNS disease occurring within 30 days of a systemic infection/vaccination; b) the identification of inflammatory findings on MRI and/or CSF analysis; c) the exclusion of alternative conditions like NMOSD, Bickerstaff/Miller-Fisher syndromes, infectious encephalitis, vasculitis and MS as well as other rarer conditions.

In our cohort of consecutive patients recruited at Foundation C. Mondino in Pavia, mean age at onset of the disease was 50.1 years, and the female:male ratio was 1.4, differently from many other conditions like MS which typically affects a larger fraction of women at a younger age (ratio 2.5-3.0) (Thompson et al., 2018b). Most PINS cases affected the spinal cord, either alone (44%) or in combination with the brain (44%), with an involvement of PNS in 41% of patients.

Evidence of inflammation in CSF was detected by blood-brain barrier damage, lymphomonocytoid pleocytosis or abnormal focusing patterns, with prevalence of type III (mirror) and IV (mixed). Type II (CSF-specific OBs), when present, showed to be transient in almost 50% of cases.

Furthermore, CSF-specific OBs were found in 21% of cases differently from what happen in diseases like MS, in which around 90% of patients have CSF-specific OBs (Thompson et al., 2018b). Also MRI presentation is different from other conditions like MS: periventricular lesions were less common (31%), and involvement of corpus callosum was very rare in this cohort of patients. However, none of cases fulfilled the recent McDonald criteria for the diagnosis of MS, even among the relapsing (18%) and chronic progressive (15%) cases. In general, acute response to steroids was very good (72%), and only a minority of patients received also intravenous immunoglobulin administration. At follow-up after several years (mean of 7.2 years), half of patients were fully active (55%), and use of immunosuppressive treatments was limited to few cases.

Despite the existence of clear clinical differences between PINS and MS, in this paper we took advantage of the recent advances in the knowledge of genetic contribution to MS. As a matter of fact, the IMSGC consortium identified the existence of 200 autosomal common variants associated with MS (IMSCG, 2019). As in other complex diseases, polygenic risk scores have proven to be an effective tool for investigation of the genetic architecture and shared genetic aetiology between traits, by aggregating genetic contribution (Jostins and Barrett, 2011) of established risk loci. Therefore, in this paper we calculated a wGRS using 183 autosomal SNPs, one low-frequency SNP from Exome Chip, and HLAGB score using 7 HLA loci associated to MS. The wGRS of PINS was significantly lower than RRMS/SPMS cases, and similar to HC, and the same pattern was found with the HLAGB score. The difference was even greater for PINS with PNS involvement vs BOMS, while no influence of wGRS on clinical status at follow-up evaluation was detected.

Despite clinical differences, when we tested the predictive ability of this score to discriminate between MS and PINS, the ability was poor (AUC: 0.628), even after the addition of HLA (AUC: 0.657). It is possible that the development of more complex prediction models including not only genetic susceptibility but also environmental risk factors like serum vitamin D levels, body mass

index, cigarette smoking and viral exposures could help in the discrimination between PINS and MS, as well as the identification of a biomarker of this condition, or by inclusion of risk factors in non-additive fashion. Moreover, since we confined calculation of wGRS to loci with robust association, we cannot rule out the possibility that the adoption of a more comprehensive polygenic score for MS, by inclusion of markers that did not achieve genome-wide significance could better interrogate the genetic relationship between the two diseases: genuinely associated signals may in fact lie in the set of loci with suggestive or even nominal level of significance. At present time, we can only say that the set of genetic variants associated to MS seems not to confer a general risk of CNS inflammation, as suggested in a previous paper which compared the wGRS calculated on a limited set of MS variants of pediatric-onset MS with monophasic acquired CNS demyelination, and obtained similar results to us (van Pelt et al., 2013). Moreover, we can support the usefulness to discriminate PINS from MS cases not only from a clinical point of view, but also from an etiopathogenetic point of view.

Some drawbacks of this study must be mentioned. First of all, sample size is limited by the intrinsic rarity of PINS conditions, and also by the unawareness of some centers about this condition. Due to this limitation, we were unable to draw conclusions on the existence of similarities and differences at single variant level.

5. CONCLUSION

This study provides additional evidence that PINS syndromes should be considered and treated as a different clinical condition than MS. Additional recruitment of PINS patients through large-scale international collaborations, like the IMSGC, and more detailed genetic investigation on PINS are warranted to better understand whether this is an heterogeneous clinical condition or a separate entity.

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Declaration of Competing Interests

RC, **SS**, **GB**, **MS**, **LF**, **SP**, **EM**, **AG**, **EV**, **IC**, **AC**, **FE**, **FC** and **EM** declare no conflicts of interests.

FMB has received compensation for consulting services and/or speaking activities from Teva Pharmaceutical Industries, Sanofi Genzyme, Merck-Serono, Biogen Idec, Roche, Medday, Excemed, and received research support from Merck, Teva Pharmaceutical Industries, Italian Ministry of Health, Fondazione Italiana Sclerosi Multipla and Fondazione Cariplo.

GC received honoraria from Novartis, Teva, Sanofi Genzyme, Merck, Biogen, Roche, Celgene, Forward Pharma, Medday, Excemed.

MF is Editor-in-Chief of the *Journal of Neurology*; serves on the scientific advisory board for Teva Pharmaceutical Industries; has received compensation for consulting services and/or speaking activities from Biogen Idec, Excemed, Novartis, and Teva Pharmaceutical Industries; and receives research support from Biogen Idec, Teva Pharmaceutical Industries, Novartis, Italian Ministry of Health, Fondazione Italiana Sclerosi Multipla, Cure PSP, Alzheimer's Drug Discovery Foundation (ADDF), the Jacques and Gloria Gossweiler Foundation (Switzerland), and ARiSLA (Fondazione Italiana di Ricerca per la SLA).

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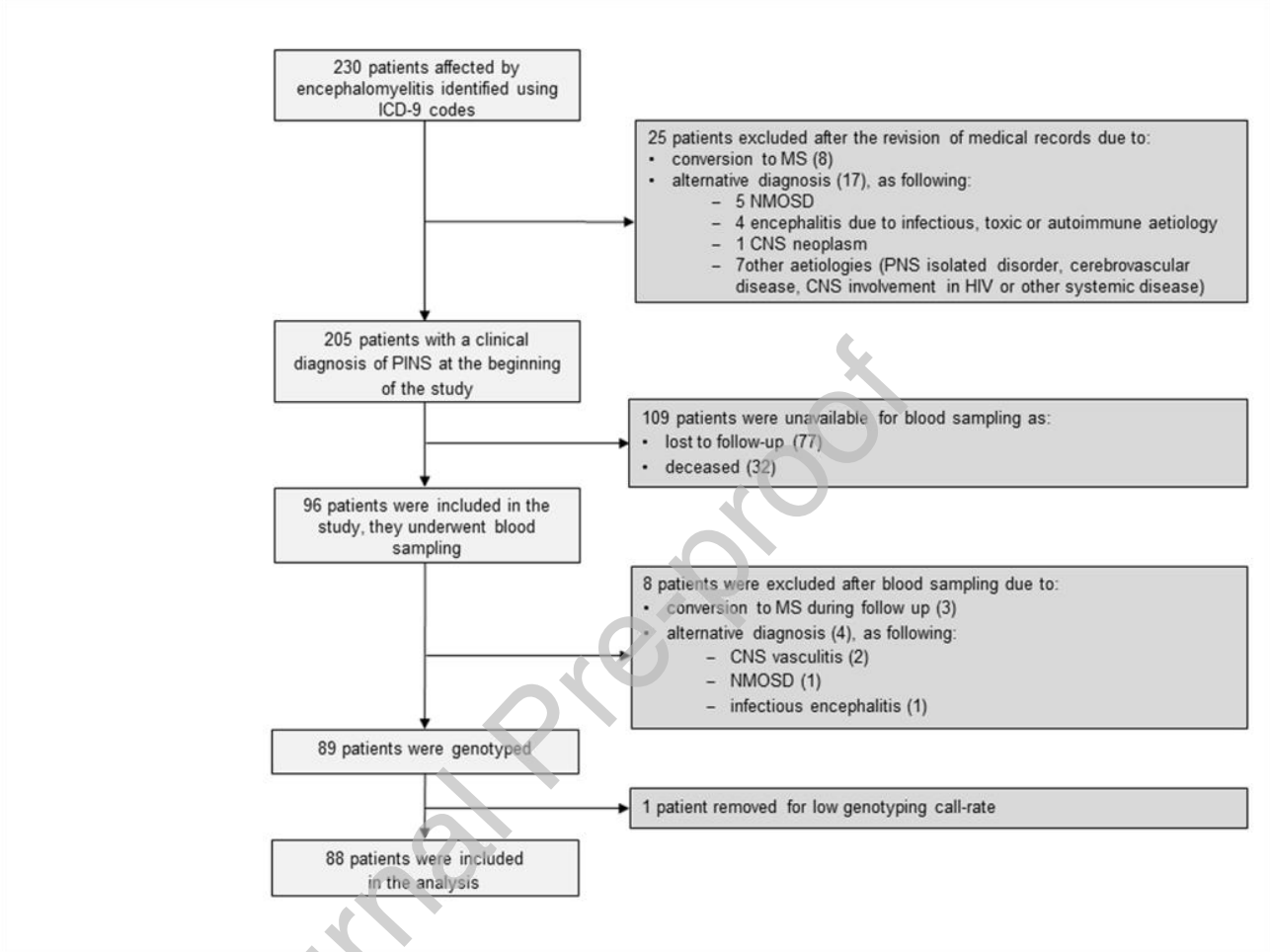
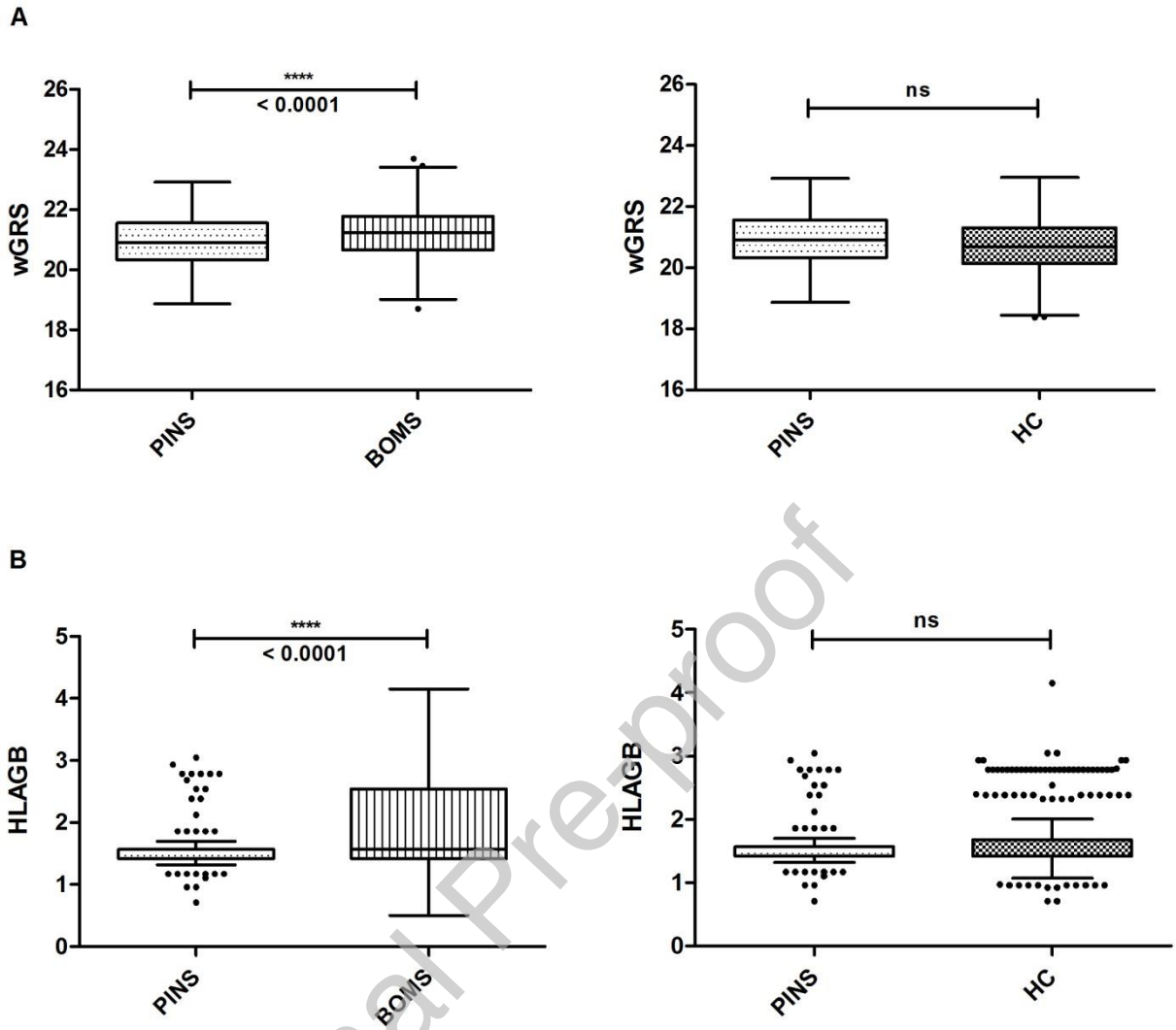
Figures and Tables

Figure 1 - Flow-chart of selection of patients for PINS cohort, considering inclusion and exclusion criteria.



Boxes in gray show the number of patients excluded from the analyses and reasons for exclusion.
PINS: Post-infectious Neurologic Syndromes, MS: Multiple Sclerosis, NMOSD: Neuromyelitis Optica Spectrum Disorders, CNS: Central Nervous System, PNS: Peripheral Nervous System, ICD-9: International Classification of Diseases.

Figure 2 - wGRS and HLAGB scores of the three analyzed cohorts are plotted using box plots.



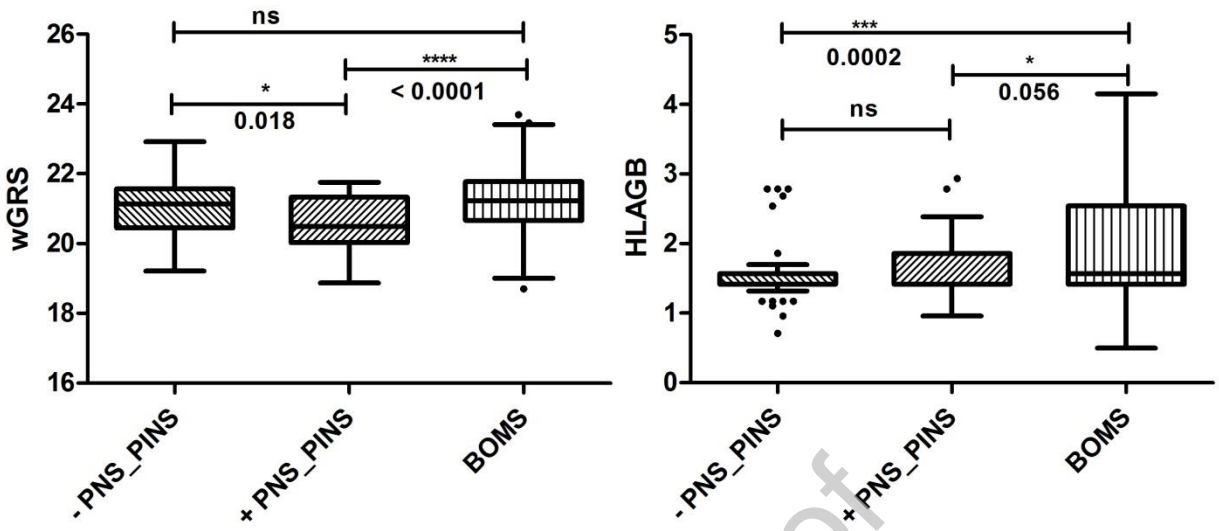
A) wGRS distribution in PINS compared to BOMS (left) and to HC (right).

B) HLAGB distribution in PINS compared to BOMS (left) and HC (right).

Horizontal lines within boxes represent the median value, boxes extend from 25th to 75th percentiles, and upper and lower whiskers are plotted according to Tukey method.

P-values were derived from t-test and Mann-Whitney U test for wGRS and HLAGB respectively (ns: not significant).

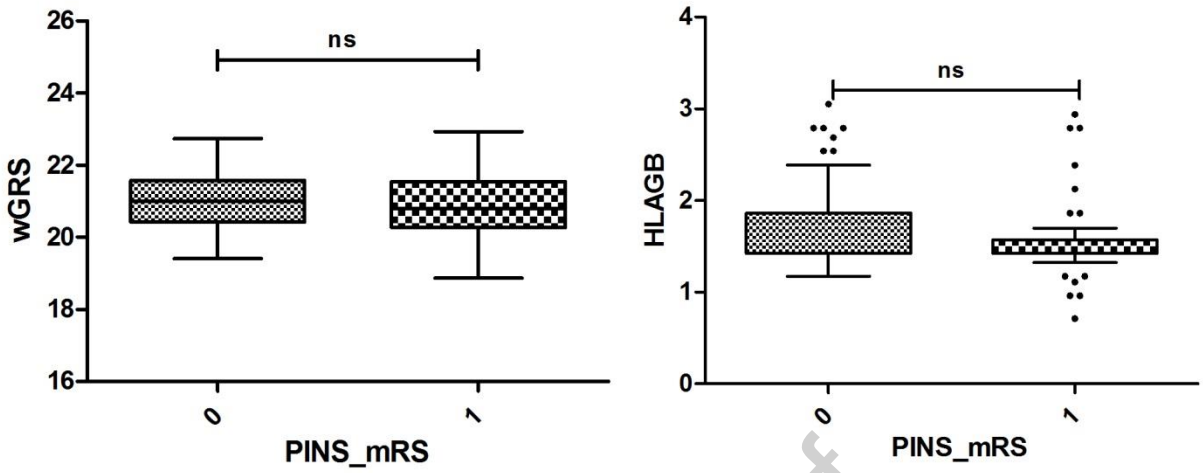
Figure 3 - Distribution of wGRS (left) and HLAGB (right) scores in PINS cohort, stratified by involvement of Peripheral Nervous System.



Scores of PINS patients with (+PNS_PINS) and without (-PNS_PINS) involvement of PNS are compared to scores in BOMS using box plots. Horizontal lines within boxes represent the median value, boxes extend from the 25th to 75th percentiles, and upper and lower whiskers are plotted according to Tukey method.

P-values were derived from t-test and Mann-Whitney U test for wGRS and HLAGB respectively (ns: not significant).

Figure 4 – Distribution of wGRS (left) and HLAGB (right) scores in PINS cohort, stratified by modified Rankin Scale.



Value of modified Rankin Scale (mRS) at follow-up visits were dichotomized (0: mRS<=1, 1: mRS>1) and distribution of scores reported with box plots. Horizontal lines within boxes represent the median value, boxes extend from the 25th to 75th percentiles, and upper and lower whiskers are plotted according to Tukey method.

P-values were derived from t-test and Mann-Whitney U test for wGRS and HLAGB respectively (ns: not significant).

Table 1. Clinical and paraclinical features of the 88 PINS patients included in the analyses

Prodromal event	
Infection	59/88 (68%)
Vaccination	4/88 (5%)
Clinical manifestations	
Myelopathy	72/88 (82%)
Encephalopathy	21/88 (24%)
Meningismus	9/88 (10%)
Seizures	3/88 (3%)
Focal deficits	2/88(2%)
Optic neuritis (mono or bilateral)	10/88 (12%)
Brainstem/cerebellar dysfunction	13/88 (15%)
Cranial nerve palsies	8/88 (9%)
PNS involvement *	28/68 (41%)
Pattern of PNS involvement	
Axonal	11/28 (39%)
Demyelinating	16/28 (57%)
Mixed	1/28 (4%)
CSF analysis	
Elevated protein levels	52/80 (70%)
Pleocytosis	32/80 (40%)
CSF-specific oligoclonal bands	16/76 (21%)

Lesion distribution on MRI	
Periventricular or subcortical white matter	27/87 (31%)
Corpus callosum	3/87 (3%)
Basal ganglia	6/87 (7%)
Brainstem/cerebellum	27/87 (31%)
Spinal cord	70/87 (80%)
Steroid administration	78/88(89%)
Response to steroids **	
Good	56/78 (72%)
Poor	22/78 (28%)
Intravenous immunoglobulin administration	16/88 (18%)
mRS at peak	
0-1	15/88 (17%)
>1	73/88 (83%)
mRS at discharge	
0-1	43/88 (49%)
>1	45/88 (51%)
Disease course	
Monophasic	59/88 (67%)
Relapsing	16/88 (18%)
Chronic progressive	13/88 (15%)

CSF= cerebrospinal fluid; MRI=magnetic resonance imaging; mRS=modified Rankin Scale; PNS=peripheral nervous system.

* disclosed or confirmed by electroneurography

** defined by a decrease of at least one point on the modified Rankin Score

Table 2. Demographic and disease features of PINS, BOMS and HC cohorts

	PINS	BOMS	HC	p value
Gender, M:F	1 : 1.4	1 : 2.1	1 : 0.49	< 0.0001
Age, mean years ± SD	56.9 ± 16.2	39 ± 10.2	45 ± 12.9	< 0.0001
Age at onset, mean years ± SD	50.1 ± 15.4	29 ± 9.1	-	< 0.0001
Disease duration, mean years ± SD	7.2 ± 4.6	11 ± 9.1	-	< 0.0001

M: male; F: female; SD: standard deviation

P-value for gender was obtained by Chi-Squared test and p value for age, age at onset and disease duration were obtained by Student T-Test.

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