SUPPLEMENTARY TABLE S1. Number of samples obtained in each hospital

	HD (n)	MS patients (n)		
Hospital	**Single Time Point**	**Baseline**	**12 months**	**24 months**
HUPHM	21	20	20	0
HUG	0	12	12	11
Total	21	32	32	11

Samples from healthy donors (HD) were obtained at a single time point. Samples from multiple sclerosis (MS) patients were obtained immediately before starting dimethyl fumarate (DMF) treatment (baseline) and after 12 and 24 months of therapy with DMF.

HUPHM, Hospital Universitario Puerta de Hierro Majadahonda; HUG, Hospital Universitario de Getafe

SUPPLEMENTARY TABLE S2. Clinical and MRI outcomes

	Time periods			Statistical significance		
	Baseline	**1 year**	**2 years**	**Baseline vs 1 year**	**1 year vs 2 years**	**Baseline vs 2 years**
ARR†,‡						
All	0.81 ± 0.81	0.50 ± 0.88	0.30 ± 0.51	**0.0014**	0.0605	**<0.0001**
Responder	0.68 ± 0.65	0.36 ± 0.66	0.20 ± 0.40	**0.0013**	**0.0313**	**<0.0001**
Non-responder	1.11 ± 1.07	0.80 ± 1.23	0.50 ± 0.67	0.2500	0.6250	0.2402
Naïve	1.19 ± 0.94	0.82 ± 1.07	0.47 ± 0.60	**0.0127**	0.0938	**0.0016**
Previously treated	0.38 ± 0.26	0.13 ± 0.35	0.10 ± 0.28	**0.0494**	>0.9999	**0.0085**
Women	0.72 ± 0.64	0.29 ± 0.62	0.27 ± 0.55	**0.0001**	0.5625	**0.0003**
Men	1.10 ± 1.20	1.13 ± 1.25	0.38 ± 0.35	>0.9999	0.0625	0.1484
EDSS†						
All	1.30 ± 1.31	1.14 ± 1.25	1.11 ± 1.35	0.0742	0.6523	0.0829
Responder	0.95 ± 0.90	0.82 ± 0.84	0.75 ± 0.94	0.0625	0.4375	0.0781
Non-responder	2.05 ± 1.77	1.85 ± 1.70	1.90 ± 1.79	0.5000	>0.9999	0.6719
Naïve	1.06 ± 1.06	0.85 ± 0.86	0.88 ± 0.93	0.0625	>0.9999	0.3438
Previously treated	1.57 ± 1.55	1.47 ± 1.54	1.37 ± 1.71	0.6250	0.4375	0.1719
Women	1.25 ± 1.42	1.06 ± 1.35	1.13 ± 1.48	**0.0156**	0.5938	0.3389
Men	1.44 ± 0.98	1.38 ± 0.92	1.06 ± 0.94	>0.9999	0.2500	0.2500
Number of GdE lesions†						
All	0.53 ± 0.92	0.00 ± 0.00	0.13 ± 0.55	**0.0010**	0.5000	**0.0303**
Responder	0.45 ± 0.91	0.00 ± 0.00	0.00 ± 0.00	**0.0313**	>0.9999	**0.0313**
Non-responder	0.70 ± 0.95	0.00 ± 0.00	0.40 ± 0.97	0.0625	0.5000	0.5625
Naïve	0.82 ± 1.13	0.00 ± 0.00	0.06 ± 0.93	**0.0078**	>0.9999	**0.0078**
Previously treated	0.20 ± 0.41	0.00 ± 0.00	0.20 ± 0.77	0.2500	>0.9999	>0.9999
Women	0.38 ± 0.71	0.00 ± 0.00	0.00 ± 0.00	**0.0156**	>0.9999	**0.0156**
Men	1.00 ± 1.31	0.00 ± 0.00	0.50 ± 1.07	0.1250	0.5000	0.5000
Number of new T2-weighted lesions†,§						
All	1.27 ± 0.59	0.44 ± 1.05	0.28 ± 0.89	**0.0002**	0.3438	**0.0083**
Responder	1.18 ± 0.40	0.25 ± 0.85	0.04 ± 0.20	**0.0010**	0.2500	**0.0010**
Non-responder	1.50 ± 1.00	1.00 ± 1.41	1.00 ± 1.60	0.5000	>0.9999	>0.9999
Naïve	-	0.64 ± 1.32	0.35 ± 1.00	-	0.3125	-
Previously treated	1.27 ± 0.59	0.20 ± 0.56	0.20 ± 0.77	**0.0002**	>0.9999	**0.0083**
Women	1.36 ± 0.50	0.33 ± 0.92	0.08 ± 0.28	**0.0020**	0.2500	**0.0010**
Men	1.00 ± 0.82	0.75 ± 1.39	0.88 ± 1.64	0.2500	>0.9999	0.7500
Percentage of relapse-free patients¶						
All	46.88	90.63	93.75	OR= 0.09 (95% IC: 0.02-0.35) **p=0.0002**	OR= 0.64 (95% IC: 0.11-3.37) p=0.6414	OR= 0.06 (95% IC:0.01-0.029) **p<0.0001**
Responder	54.55	100	100	OR= 0.00 (95% IC: 0.00-0.25) **p=0.0003**	-	OR= 0.00 (95% IC: 0.00-0.25) **p=0.0003**
Non-responder	30	70	70	OR= 0.18 (95% IC: 0.04-1.48) p=0.0736	OR= 1.00 (95% IC: 0.18-5.519) p>9999	OR= 0.18 (95% IC: 0.04-1.48) p=0.0736
Naïve	11.76	88.24	94.12	OR= 0.02 (95% IC: 0.00-0.14) **p<0.0001**	OR= 0.47 (95% IC: 0.03-4.45) p=0.5454	OR= 0.01 (95% IC: 0.00-0.10) **p<0.0001**
Previously treated	86.67	93,33	93.33	OR= 0.46 (95% IC: 0.03-4.47) p=0.5428	OR= 1.00 (95% IC: 0.05-20.23) p>0.9999	OR= 0.46 (95% IC: 0.03-4.47) p=0.5428
Women	50	95.83	95.83	OR= 0.04 (95% IC: 0.00-0.33) **p=0004**	OR= 1.00 (95% IC: 0.05-19.74) p>0.9999	OR= 0.04 (95% IC: 0.00-0.33) **p=0004**
Men	37.5	75	87.50	OR= 0.20 (95% IC: 0.03-1.63) p=0.1306	OR= 0.43 (95% IC: 0.03-4.64) p=0.5218	OR= 0.09 (95% IC: 0.01-1.00) **p=0.0389**
Percentage of CDP-free patients¶						
All	-	96.88	87.50	-	OR= 4.43 (95% IC: 0.65-55.67) p=0.1623	-
Responder	-	100	100	-	-	-
Non-responder	-	90	70	-	OR= 3.86 (95% IC: 0.45-54.81) p=0.2636	-
Naïve	-	100	88.24	-	OR= ∞ (95% IC: 0.47-∞) p=0.1449	-
Previously treated	-	93.75	87.50	-	OR= 2.14 (95% IC: 0.22-32.86) p=0.5442	-
Women	-	100	83.33	-	OR= ∞ (95% IC: 1-∞) **p=0.0367**	-
Men	-	87.50	100	-	OR= 0.00 (95% IC: 0.00-9.00) p=0.3017	-

Percentage of patients with GdE lesions¶						
All	34.38	0	6.25	OR= ∞ (95% IC: 4.27-∞) **p=0.0003**	OR= 0.00 (95% IC: 0.00-2.14) p=0.1508	OR= 7.86 (95% IC: 1.79-37.32) **p=0.0052**
Responder	27.27	0	0	OR= ∞ (95% IC: 2.18-∞) **p=0084**	-	OR= ∞ (95% IC: 2.18-∞) **p=0084**
Non-responder	50	0	20	OR= ∞ (95% IC: 1.70-∞) **p=0.0098**	OR= 0.00 (95% IC: 0.00-2.09) p=0.1360	OR= 4.00 (95% IC: 0.66-24.38) p=0.1596
Naïve	47.06	0	5.88	OR= ∞ (95% IC: 2.80-∞) **p=0.0012**	OR= 0.00 (95% IC: 0.00-9.00) p=0.3101	OR= 14.22 (95% IC: 1.83-165.7) **p=0.0065**
Previously treated	20	0	6.67	OR= ∞ (95% IC: 0.93-∞) p=0.0679	OR= 0.00 (95% IC: 0.00-9.00) p=0.3091	OR= 3.50 (95% IC: 0.45-48.22) p=0.2827
Women	29.17	0	0	OR= ∞ (95% IC: 2.02-∞) **p=0.0042**	-	OR= ∞ (95% IC: 2.02-∞) **p=0.0042**
Men	50	0	25	OR= ∞ (95% IC: 1.00-∞) **p=0.0209**	OR= 0.00 (95% IC: 0.00-2.07) p=0.1306	OR= 3.00 (95% IC: 0.35-20.32) p=0.3017
Percentage of patients with T2w lesions§,¶						
All	93.75	21.88	12.50	OR= 53.57 (95% IC: 6.48-581.4) **p<0.0001**	OR= 1.96 (95% IC: 0.51-6.49) p=0.3202	OR= 105.00 (95% IC: 10.42-1128) **p<0.0001**
Responder	100	9.09	0	OR= ∞ (95% IC: 20.00-∞) **p<0.0001**	OR= ∞ (95% IC: 0.47-∞) p=0.1478	OR= ∞ (95% IC: 43.62-∞) **p<0.0001**
Non-responder	75	50	40	OR= 3.00 (95% IC: 0.31-45.71) p=0.3932	OR= 1.50 (95% IC: 0.30-8.53) p=0.6531	OR= 4.50 (95% IC: 0.46-66.62) p=0.2367
Naïve	-	29.41	17.65	-	OR= 1.94 (95% IC: 0.36-8.40) p=0.4187	-
Previously treated	93.33	13.33	6.67	OR= 91.00 (95% IC: 7.28-1006) **p<0.0001**	OR= 2.15 (95% IC: 0.22-33.15) p=0.54288	OR= 196.0 (95% IC: 10.22-2124) **p<0.0001**
Women	100	16.67	8.33	OR= ∞ (95% IC: 9.95-∞) **p<0.0001**	OR= 2.20 (95% IC: 0.46-12.34) p=0.3827	OR= ∞ (95% IC: 22.17-∞) **p<0.0001**
Men	75	37.50	25	OR= 5.00 (95% IC: 0.46-75.79) p=0.2207	OR= 1.80 (95% IC: 0.26-12.87) p=0.5896	OR= 9.00 (95% CI: 0.73-132.1) p=0.0977

Clinical and MRI variables were measured before starting DMF treatment (baseline) and after 12 and 24 months of therapy in MS patients. Statistical significance was assessed before treatment and after 1 and 2 years in the whole sample and in the 3 subgroups of patients. $p<0.05$ was considered statistically significant.

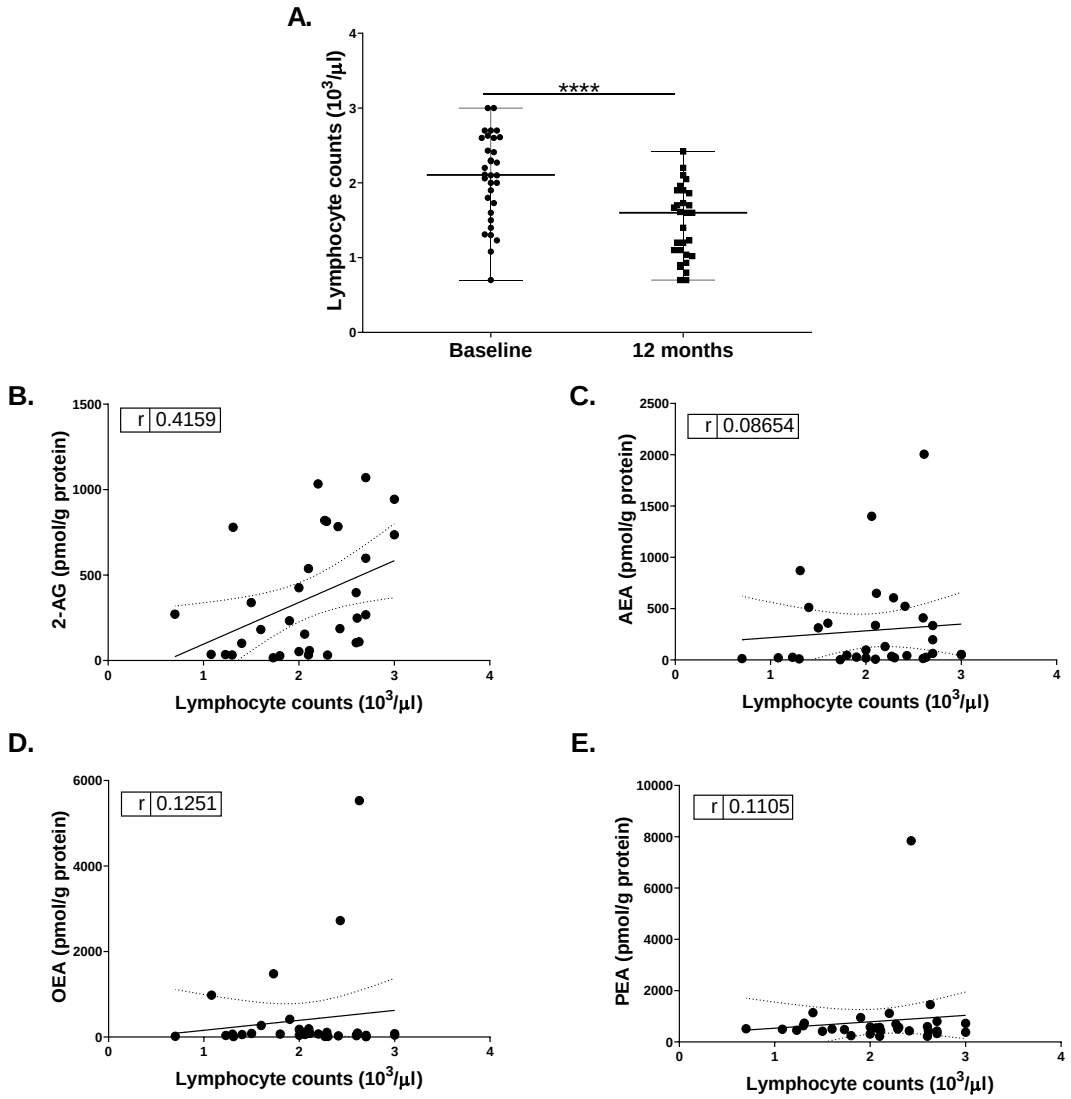
†For numerical variables, p-values were calculated using the Wilcoxon signed rank test. Values are the mean +/- SD.

‡For baseline ARR, only the previous year was considered.

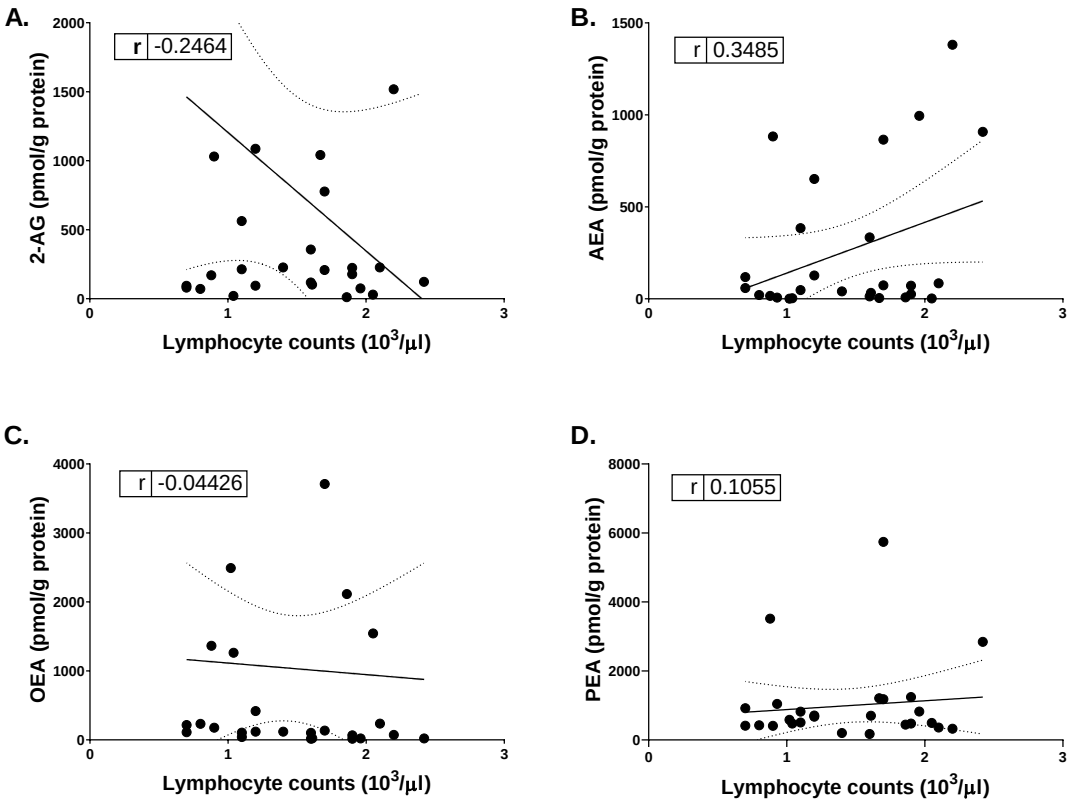
§ Data not available for naïve MS patients at baseline, as most of them had a single magnetic resonance imaging (MRI).

¶ For percentages, odds ratio, confidence interval and p-values were calculated using the Chi-square test.

ARR, annualized relapse rate; EDSS, expanded disability status scale; GdE, gadolinium-enhanced T1 lesions; CDP, confirmed disease progression, NEDA-3, no evidence of disease activity 3

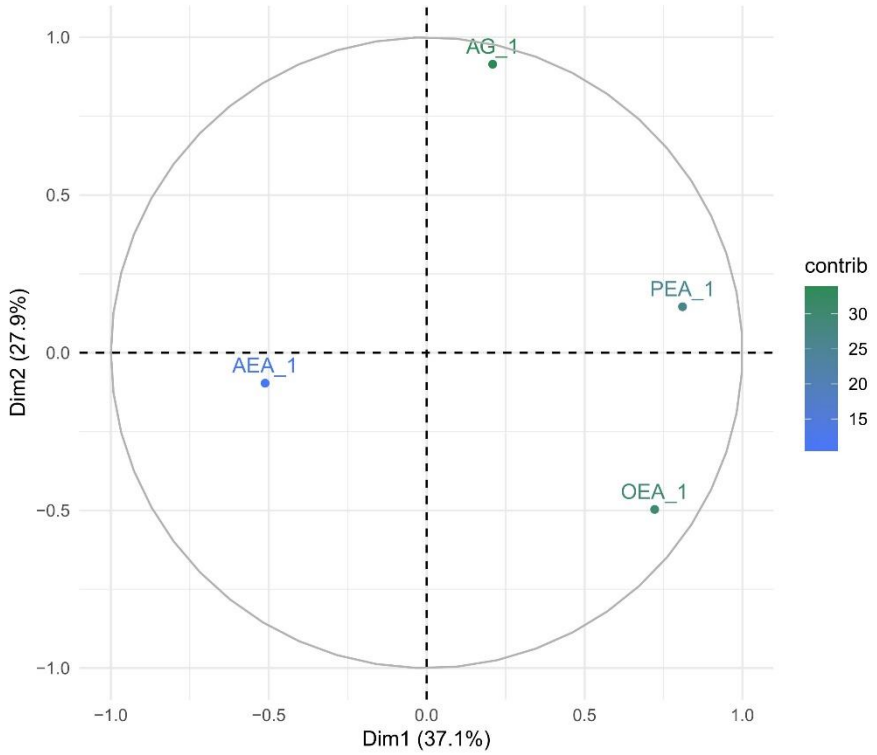


SUPPLEMENTARY FIGURE S1. Correlations between absolute lymphocyte counts and endocannabinoids at baseline. A. Lymphocyte counts at baseline and after 12 months of DMF therapy. Wilcoxon test was used to assess statistical significance. **B.** Correlation of 2-AG at baseline with lymphocyte counts at baseline. **C.** Correlation of AEA at baseline with lymphocyte counts at baseline. **D.** Correlation of OEA at baseline with lymphocyte counts at baseline. E. Correlation PEA at baseline with lymphocyte counts at baseline. B-E The Pearson correlation coefficient was calculated. ****p<0.0001



SUPPLEMENTARY FIGURE S2. Correlations between absolute lymphocyte counts and endocannabinoids at 12 months. A. Correlation of 2-AG at 12 months with lymphocyte counts at 12 months. **B.** Correlation of AEA at 12 months with lymphocyte counts at 12 months. **C.** Correlation of OEA at 12 months with lymphocyte counts at 12 months. **D.** Correlation PEA at 12 months with lymphocyte counts at 12 months. **B-E** The Pearson correlation coefficient was calculated.

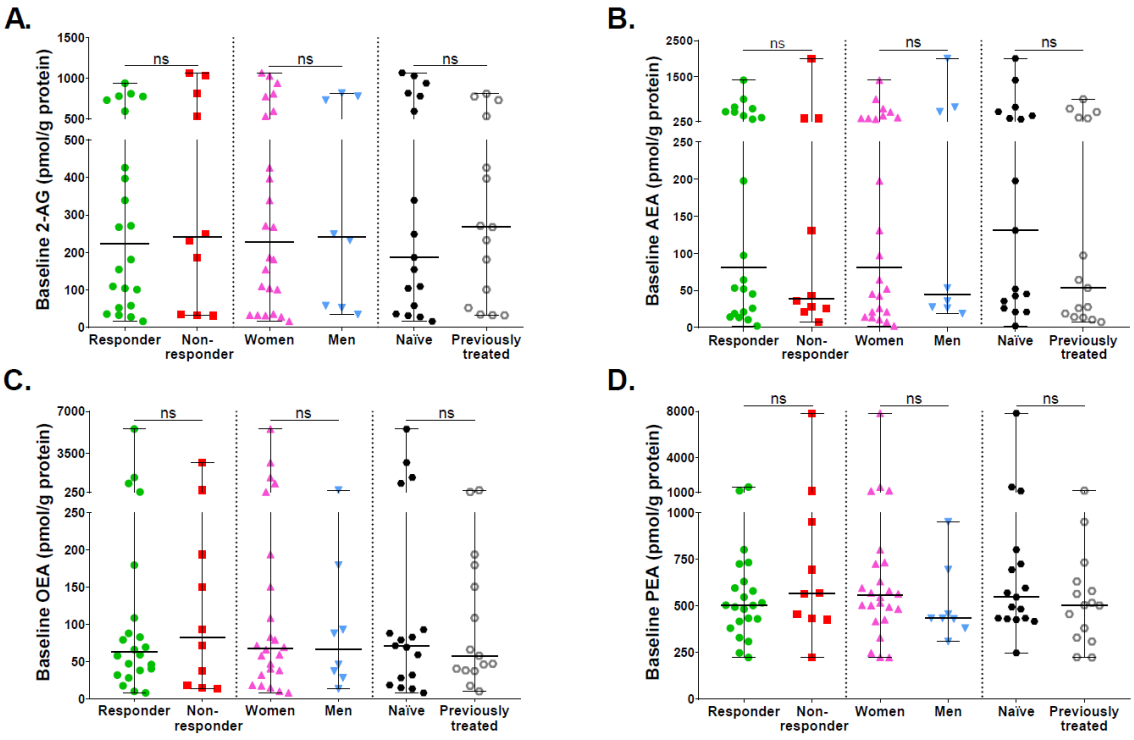
A.



B.

	Dim. 1	Dim. 2
2-AG	2,941636	75,096820
AEA	17,653538	0,838203
OEA	35,138957	22,155966
PEA	44,265868	1,909011

SUPPLEMENTARY FIGURE S3. PCA variables contribution. A. Plot showing the relationship between the four ECBs. 2-AG, OEA and PEA are the variables more distant from the origin, meaning a high contribution to the PCA. 2-AG and PEA are positively correlated variables as they are grouped together in the same quadrant. Negatively correlated ECBs are positioned on opposite sides of the plot origin (opposed quadrants). **B.** Table showing the contribution of each ECB to the dimension 1 and 2 of the PCA. Variables that are correlated with Dim.1 and Dim.2 are the most important in explaining the variability in the data set.



SUPPLEMENTARY FIGURE S4. Endocannabinoid levels at baseline in the subgroups of MS patients. (A-D) 2-AG, AEA, OEA and PEA baseline levels (in pmol/g of protein) in responders vs non-responders, women vs men and naïve vs previously treated patients. No differences were found for any of the ECBs in any of the subgroups. The Mann-Whitney test was used to compare differences between subgroups. Data are expressed as median with range. ns, not significant