Evaluation of the coagulation dysfunction in Multiple Sclerosis from the perspective of IgG antibodies against thrombus-related components and genetic polymorphisms

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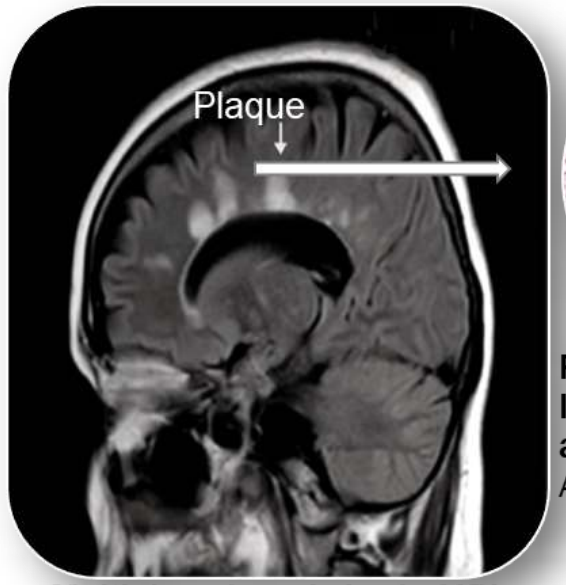
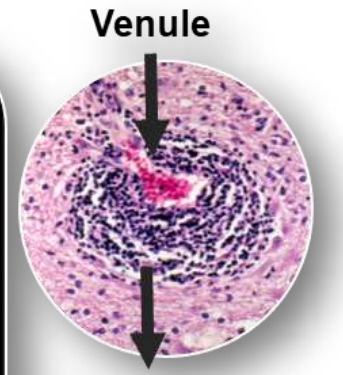
Multiple Sclerosis - Overview

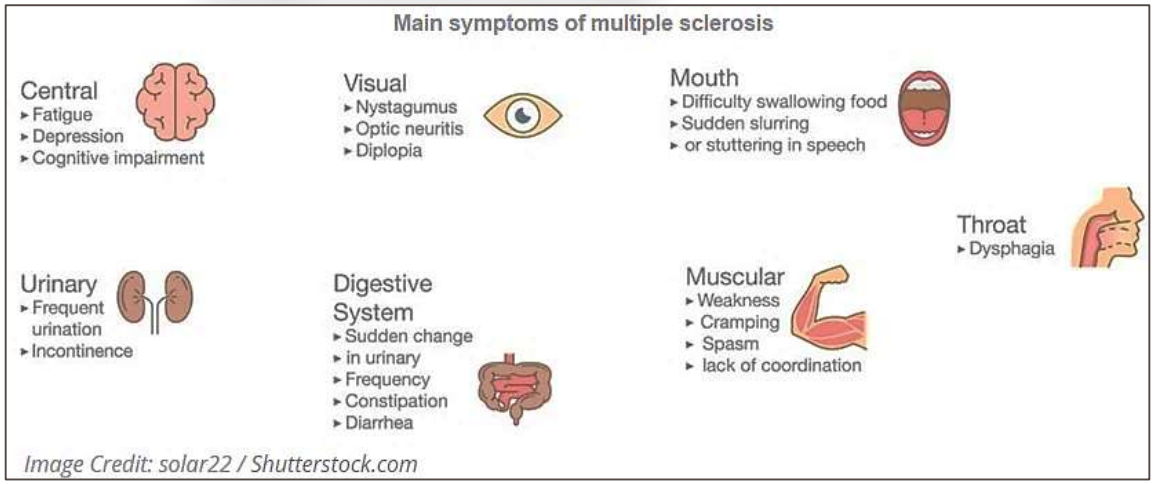
- An idiopathic autoimmune inflammatory disease of the central nervous system (CNS) presenting a relapsing or progressive course
- Leading cause of no traumatic neurological disability in young adults
- Characterized by disseminated lesions or “plaques” within the CNS due to inflammation and demyelination
- Immensely variable symptomatology, including impaired vision, decreased sensation, paresthesia, ataxia, motor weakness and imbalance



Multiple sclerosis brain MRI



Perivascular lymphocytes in an active MS plaque.
Agamanolis D. (2015)



Multiple Sclerosis - Pathology

- Activated lymphocytes and innate immune cells in the periphery gain entry into the CNS crossing the blood-brain barrier
- In the presence of myelin antigens immune cells are re-activated *in situ* along with CNS resident cells like microglia and astrocytes
- Pro-inflammatory cytokines promote inflammation and myelin damage along with increased barrier permeability and chemotaxis of peripheral immune cells

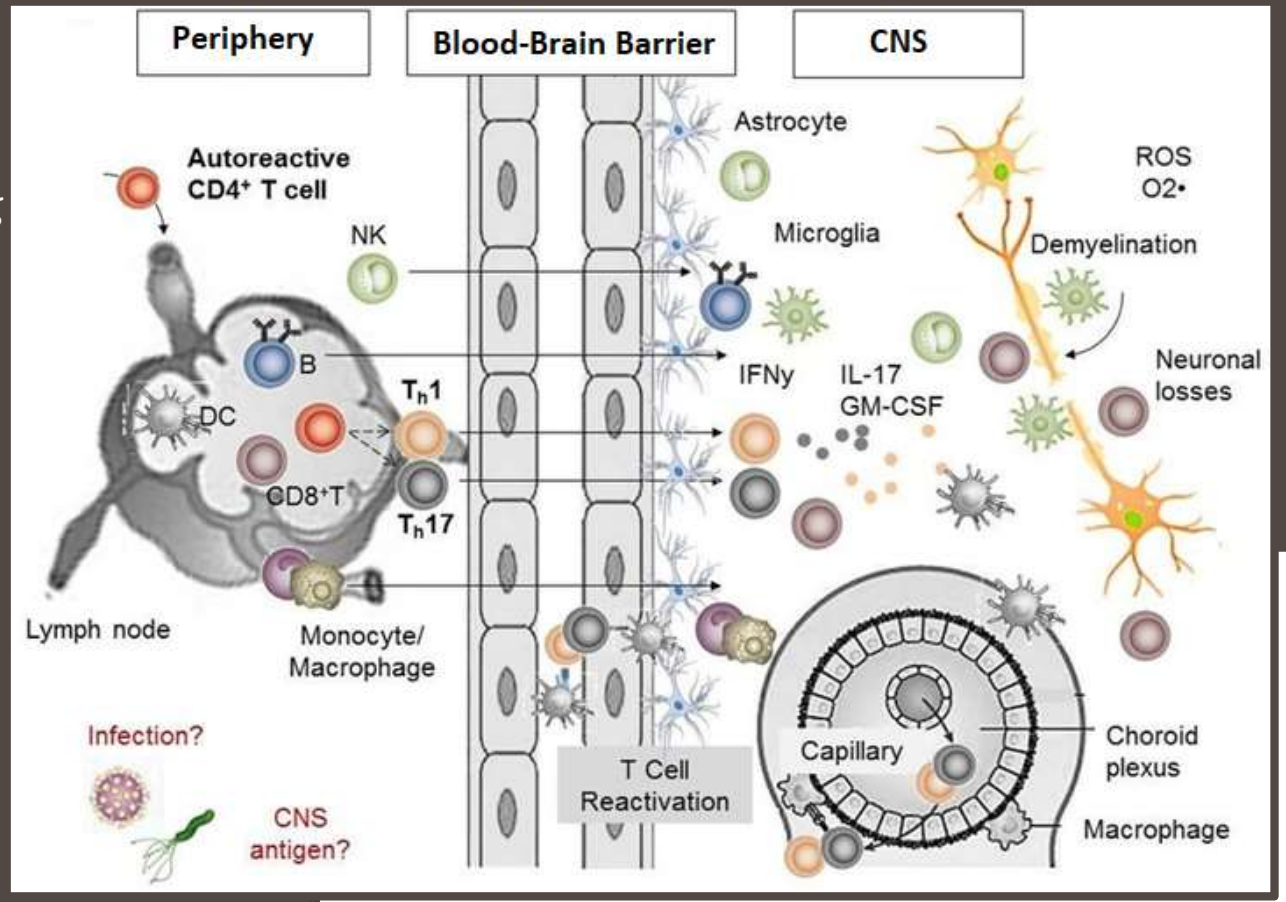
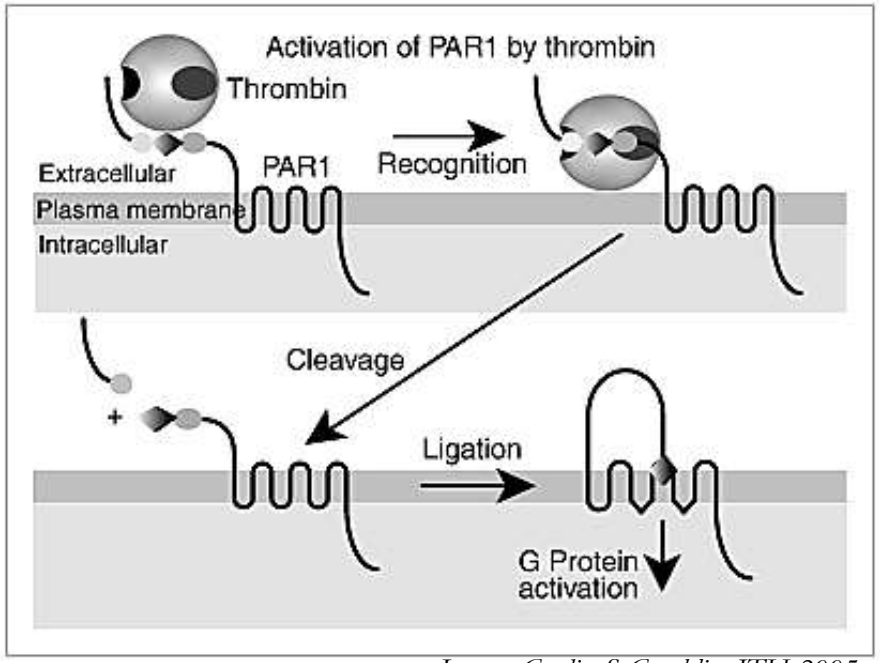


Image Credit: C. Dendrou et al., Nature 2015

Coagulation-Inflammation interplay in Multiple Sclerosis

Thrombin and fibrin(ogen)

- Modify the microglia motility towards pre-demyelinated areas
- Act as chemoattractant for the immune cells
- Induce the release of pro-inflammatory cytokines
- Enhance the blood-brain barrier disruption
- Contribute in the axonal damage and neurodegeneration



Images Credit: S.Cooghlin, JTH 2005

Thrombin's proinflammatory effects are mediated by the protease-activated receptors (PARs).

Antibodies against coagulation components in autoimmune diseases

The Journal of Immunology
RESEARCH ARTICLE | DECEMBER 15 2001

Identification of Anti-Thrombin Antibodies in the Antiphospholipid Syndrome That Interfere with the Inactivation of Thrombin by Antithrombin[1]

Kwan-Ki Hwang; ... et. al

J Immunol (2001) 167 (12):
https://doi.org/10.4049/jim
Published in final edited form as:
J Immunol. 2006 December 1; 177(11): 8219–8225.

Antibodies Against the Activated Coagulation Factor X (FXa) in the Antiphospholipid Syndrome that Interfere With the FXa Inactivation by Antithrombin

The Journal of Immunology
RESEARCH ARTICLE | FEBRUARY 01 2009

Novel Autoantibodies against the Activated Coagulation Factor IX (FIXa) in the Antiphospholipid Syndrome That Interpose the FIXa Regulation by Antithrombin[1]

Artim-Esen *et al. Arthritis Research & Therapy* (2015) 17:47
DOI 10.1186/s13075-015-0568-7
arthritis research&therapy

RESEARCH ARTICLE Open Access

Anti-factor Xa antibodies in patients with antiphospholipid syndrome and their effects upon coagulation assays

Bahar Artim-Esen[1,2*], Charis Pericleous[1], Ian Mackie[3], Vera M Ripoll[1], David Latchman[4], David Isenberg[1], Anisur Rahman[1], Yiannis Ioannou[5] and Ian Giles[1]

- **Interfere with negative feedback regulation of thrombin in circulation, contributing to thrombosis.**
- **Suppress the inactivation of the target coagulant serine proteases by antithrombin.**
- **Inhibit the enzyme activities of the target proteases in fibrinolysis.**
- **Promote thrombin-mediated activation of the complement C3 and C5.**

Brief communication
LUPUS SCIENCE & MEDICINE

Antibodies to FXa and thrombin in patients with SLE differentially regulate C3 and C5 cleavage

Thomas McDonnell [1], Raj Amarnani,[2] Carina Spicer,[3] Hajar Jbari,[2] Charis Pericleous,[4] Valentina A Spiteri,[5] Chris Wincup [2], Bahar Artim-Esen,[6] Ian Mackie,[7] Marina Botto,[4,8] Anisur Rahman [2] Ian Giles[2]

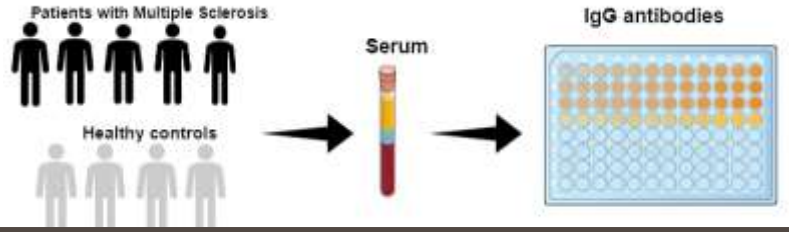
Rationale of the study

Antibodies against coagulation components seem to play a pivotal role in overlapping diseases with Multiple Sclerosis, contributing to the coagulation-inflammation circuit.

Goal and Aims

- Detection and characterization of IgG antibodies against coagulation components in patients with MS.
- Assessing the IgG levels based on patients clinical profile.
- Evaluation of the expression levels of pro-inflammatory mediators produced *in vitro* after the cell exposure to IgG antibodies studied.

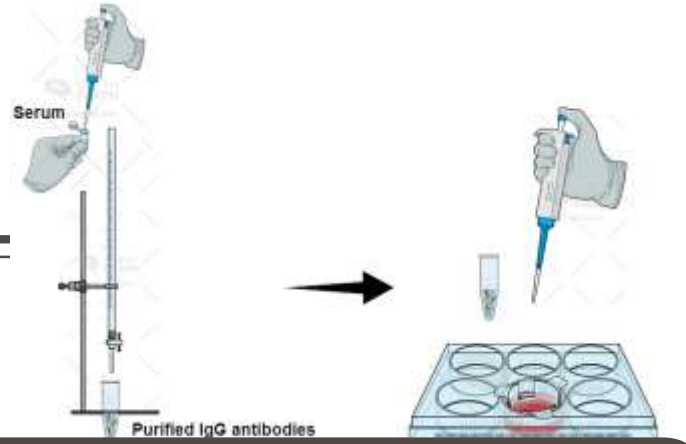
Methodology



Step 1

Screening for IgG antibodies against factor(F)VIIa, thrombin, prothrombin, FXa, FXII, plasmin, and protein C (PC).

Sera from patients with MS (n=167) and healthy controls (n=40) were analyzed using the enzyme-linked immunosorbent assay.



Step 2

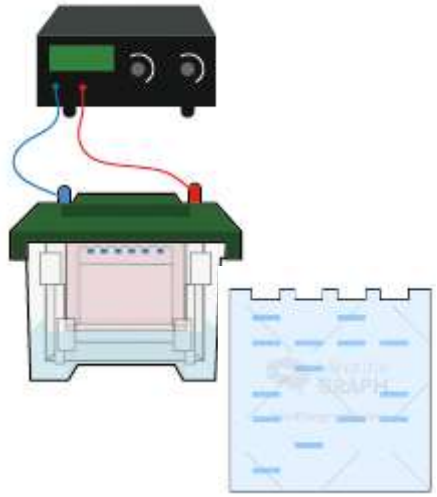
Antibody purification by affinity chromatography and endotoxin removal from purified IgG fractions

***In vitro* and *ex vivo* exposure of human astrocytes (U87 cell line and primary cells) in 100µg/ml IgG fractions**

Purified IgG samples were isolated from 15 MS patients positive for IgG tested, 8 MS negative patients, and 14 HCs.

Immunoblotting

Evaluation and quantification of the expression levels of thrombin receptor-1 (PAR-1) and phosphorylated ERK1/2 kinases



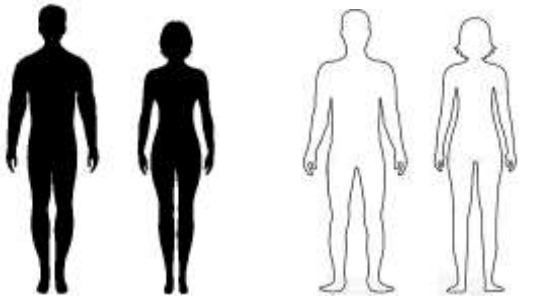
Step 3

Results

Demographics and clinical data of study participants

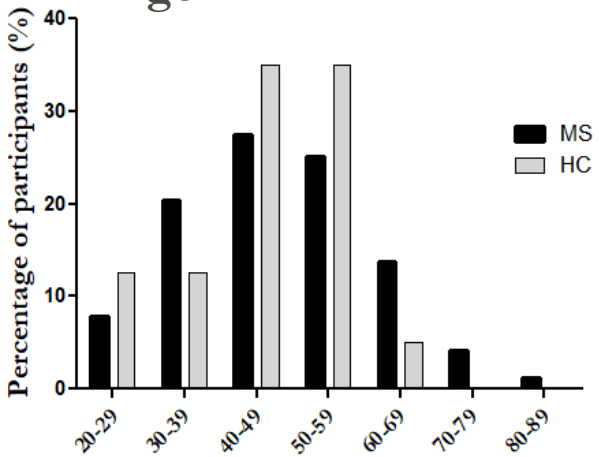
Sex

MS group HC group



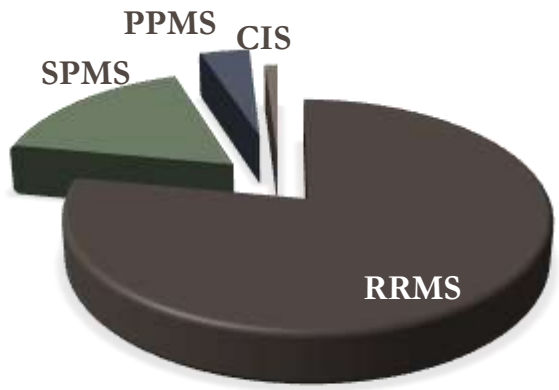
n=110 n=57 n=21 n=19

Age

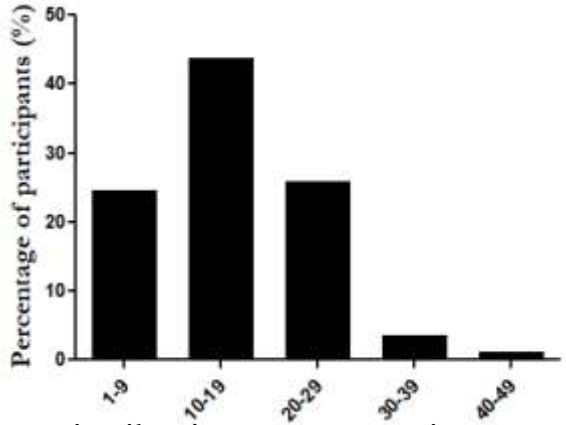


Distribution among participants

Disease courses

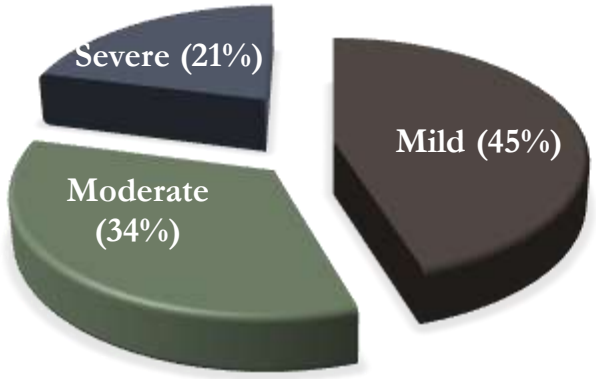


Disease duration



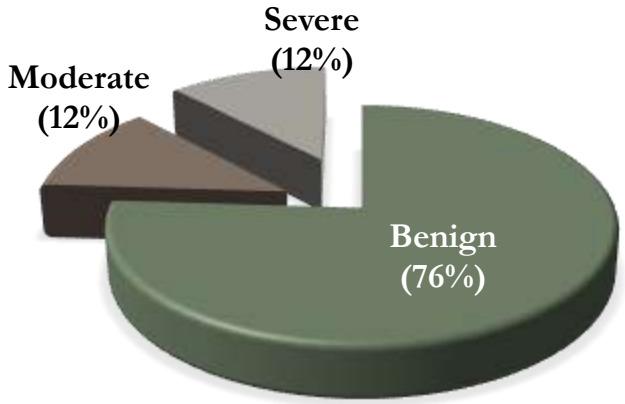
Distribution among patients

EDSS score



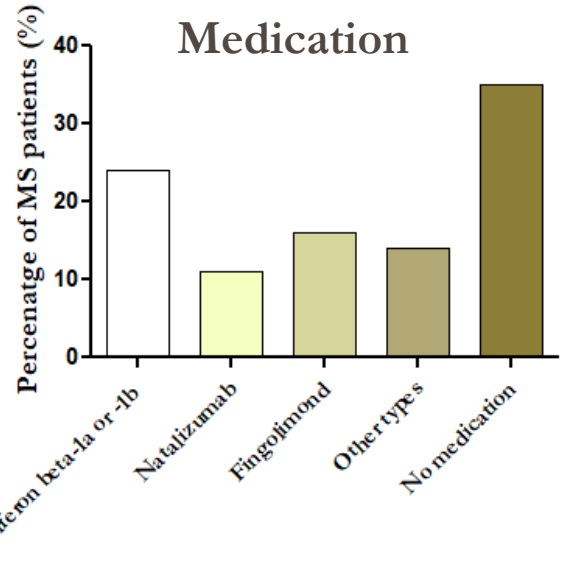
Mild: 0-3.0
Moderate: 3.5-5.5
Severe: 6.0-9.5

MS severity score



Benign: 0-1.99
Moderate: 2.0-6.99
Severe: 7.0-10

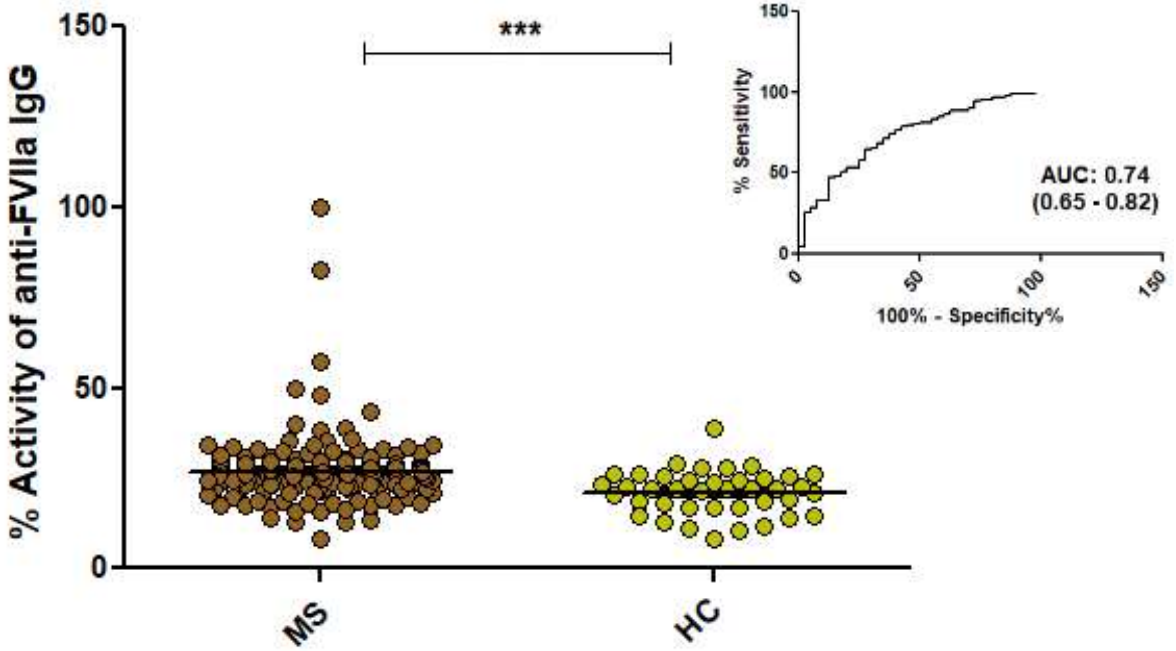
Medication

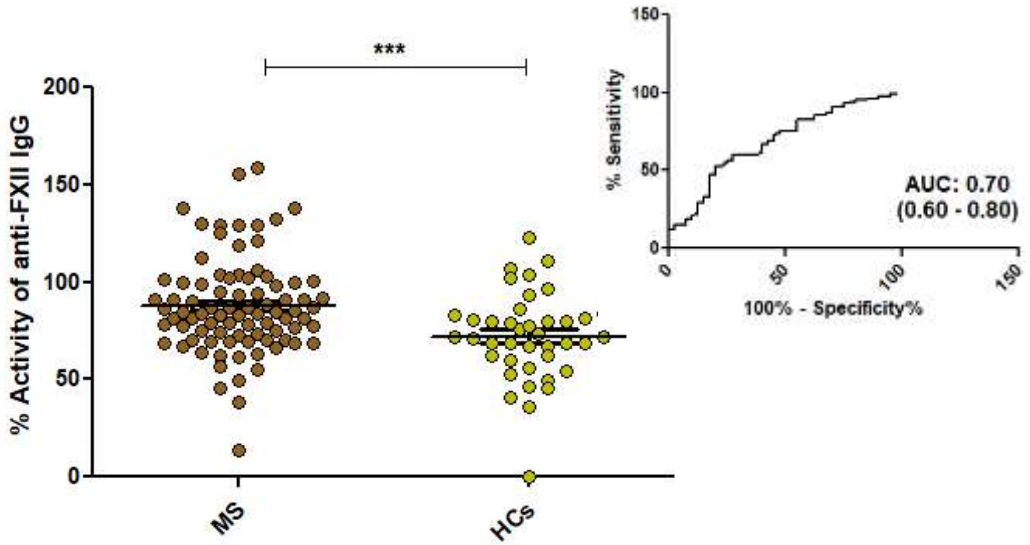


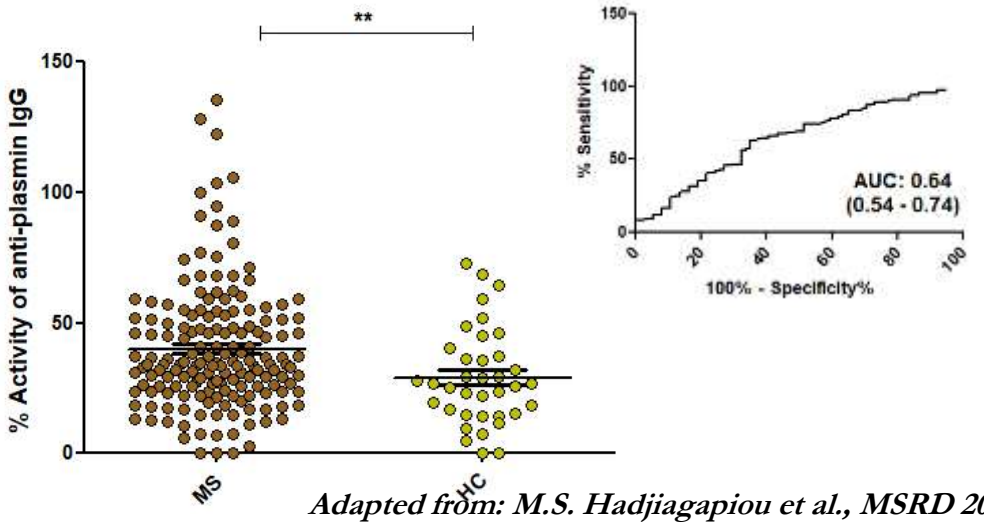
Results

Antibodies to coagulation components are implicated in MS

I. IgG antibody presence in MS patients and controls







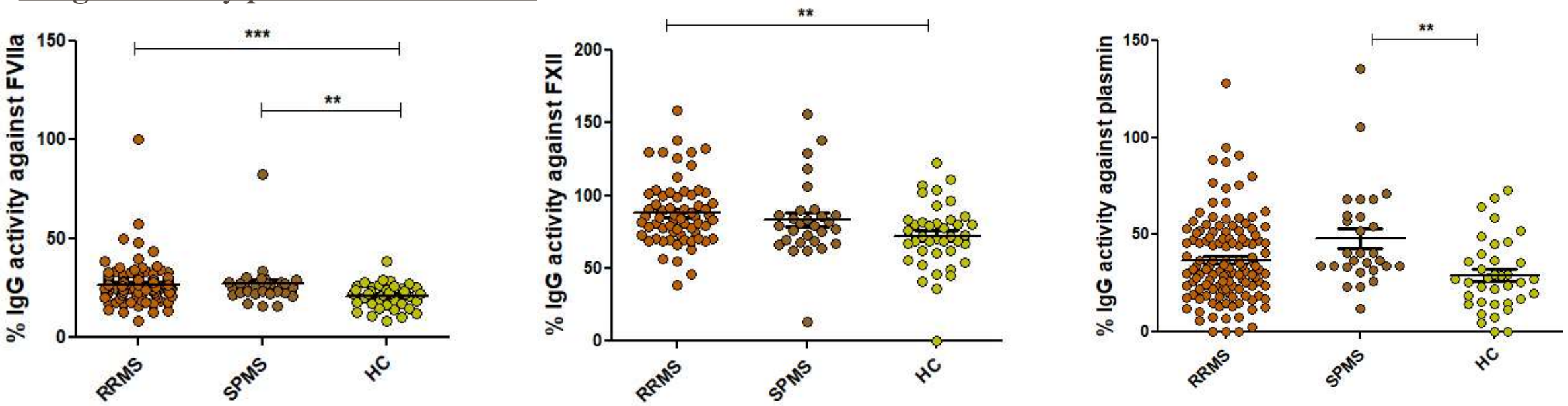
- Higher activity levels of anti-FVIIa, anti-FXII and anti-plasmin IgG antibodies in patients compared to controls.
- The presence of such factors can discriminate patients with MS from healthy controls (AUC: 0.64 – 0.74).

Adapted from: M.S. Hadjiagapiou et al., MSRD 2022

Results

Antibodies to coagulation components are implicated in MS

II. IgG antibody presence and disease courses



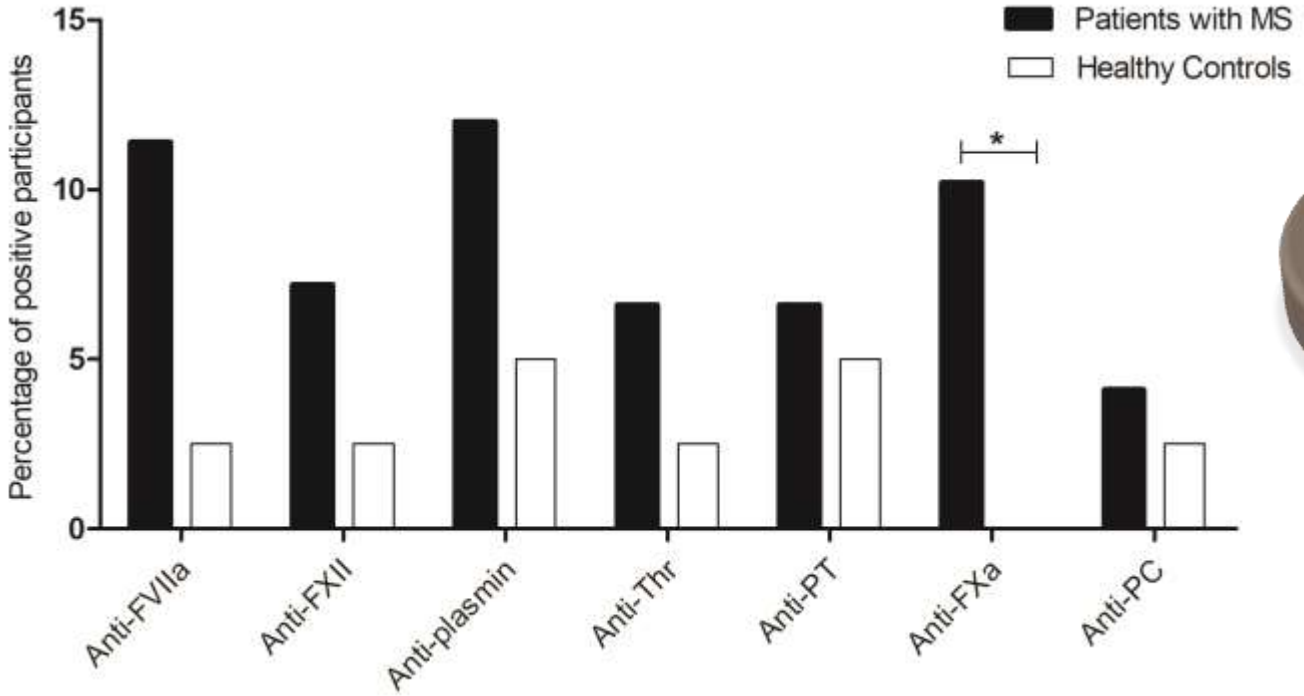
- Both RRMS and SPMS patients showed significantly higher activity to FVIIa in comparison to controls.
- RRMS patients displayed significantly increased levels of anti-FXII IgG antibodies compared to controls.
- SPMS patients exhibited a greater index of activity against plasmin that significantly differed from controls.

Adapted from: M.S. Hadjiagapiou et al., MSRD 2022

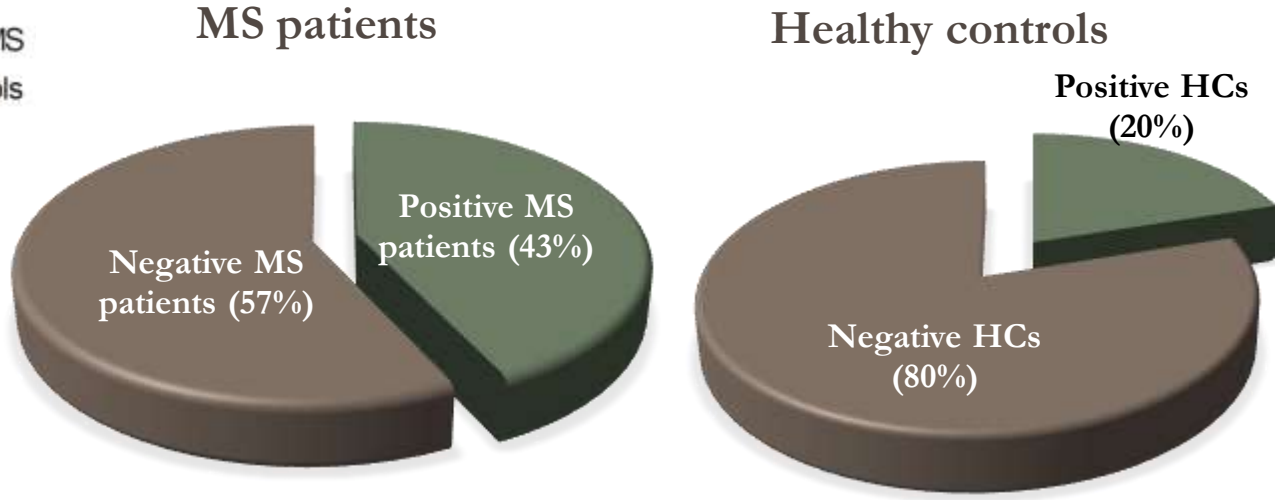
Results

IgG seropositivity in patients with MS

I. Distribution of IgG antibody positivity between MS patients and HCs



II. IgG positivity between MS patients and HCs



- Increased seropositivity for almost all the antibodies studied. Significant difference was revealed between patients and controls for the anti-FXa IgG antibodies ($p<0.05$).
- Increased proportion of patients with MS tested positive for IgG antibodies (n=72, 43%) compared to healthy controls (n=8, 20%, $p<0.001$).

Results

Association between the IgG antibodies and the clinical outcomes of MS

Correlations between IgG antibodies and demographic or clinical features of MS

	Anti-FVIIa	Anti-thrombin	Anti-prothrombin	Anti-FXa	Anti-FXII	Anti-plasmin	Anti-protein C
	Odds Ratio (95% confidence interval)						
Age	0.97 (0.94-1.01)	1.0 (0.95-1.05)	**0.95 (0.89-0.99) p= 0.03 (*)**	0.99 (0.95-1.03)	1.0 (0.95-1.05)	**1.04 (1.0-1.08) p= 0.03 (*)**	1.02 (0.95-1.05)
Gender (m=0, f=1)	0.68 (0.26-1.86)	2.22 (0.54-15.0)	0.93 (0.27-3.67)	1.78 (0.59-6.55)	0.59 (0.17-2.19)	0.59 (0.23-1.56)	3.54 (0.58-68.2)
Disease duration	0.97 (0.91-1.02)	0.97 (0.90-1.04)	**0.93 (0.85-0.99) p= 0.049 (*)**	1.0 (0.94-1.06)	1.01 (0.95-1.08)	1.03 (0.98-1.08)	0.97 (0.88-1.07)
Disease courses							
RRMS	1.07 (0.36-3.97)	0.73 (0.19-3.52)	1.23 (0.30-8.37)	0.91 (0.30-3.42)	0.59 (0.16-2.45)	0.47 (0.18-1.36)	2.67 (0.41-52.1)
SPMS	0.52 (0.08-1.99)	1.10 (0.16-4.67)	0.48 (0.02-2.72)	1.02 (0.22-3.41)	2.86 (0.67-10.89)	2.31 (0.75-6.43)	0.55 (0.03-3.62)
EDSS	0.89 (0.67-1.17)	1.35 (0.98-1.90)	0.74 (0.47-1.07)	1.22 (0.93-1.60)	0.95 (0.67-1.32	**1.30 (1.02-1.69) p= 0.03 (*)**	1.16 (0.77-1.72)
MSSS	1.0 (0.80-1.25)	**1.47 (1.10-2.02) p= 0.009 (**)**	0.92 (0.66-1.24)	1.20 (0.95-1.51)	1.17 (0.83-1.65)	1.10 (0.89-1.37)	0.95 (0.71-1.25)
Medication							
IM	0.96 (0.30-2.70)	0.28 (0.01-1.54)	0.24 (0.00-1.35)	2.06 (0.70-5.75)	0.40 (0.02-2.26)	0.64 (0.17-1.88)	1.13 (0.15-5.80)
IS	0.80 (0.27-2.16)	2.28 (0.65-8.35)	3.40 (0.98-13.47)	0.51 (0.14-1.54)	0.77 (0.19-2.70)	0.55 (0.17-1.53)	2.29(0.41-11.52)
N/M	1.01 (0.36-2.68)	0.88 (0.22-3.09)	0.64 (0.13-2.34)	0.70 (0.21-1.99)	1.94 (0.56-7.10)	2.37 (0.92-6.24)	0.41 (0.05-2.06)

*Statistically significant values (p< 0.05) are shown in bold. EDSS: Expanded Disability Status Scale; MSSS: Multiple Sclerosis Severity Score

- Antibodies against thrombin increase 47% the possibility of disease worsening.
- Anti-prothrombin IgG were less likely to be associated with the age and disease duration of MS patients.
- Patients with antibodies against plasmin had a 30% increased possibility of showing advanced disability.
- Activity against plasmin was also associated with age, indicating that as the patient gets older, there is a strong possibility these antibodies to be detected.

Adapted from: M.S. Hadjiagapiou et al., MSRD 2022

Results

In vitro optimization of astrocytic activation by IgG antibodies

Published in final edited form as:
Glia. 2020 February ; 68(2): 246–262. doi:10.1002/glia.23714.

Neuron-generated thrombin induces a protective astrocyte response via protease activated receptors

Padmesh S. Rajput[1], Jessica Lamb[1], Shweta Kothari[1], Benedict Pereira[1], Daniel Soetkamp[2], Yizhou Wang[3], Jie Tang[3], Jennifer E. Van Eyk[2], Eric S. Mullins[4], Patrick D. Lyden[1]

Astrocytes are activated by thrombin at least 2 hours after thrombin exposure (Rajput *et al.,* Glial 2020).

J Neuropathol Exp Neurol

Vol. 65, No. 1
January 2006
pp. 66–77

ORIGINAL ARTICLE

Upregulation of Protease-Activated Receptor-1 in Astrocytes in Parkinson Disease: Astrocyte-Mediated Neuroprotection Through Increased Levels of Glutathione Peroxidase

Yuri Ishida, MD, Atsushi Nagai, MD, PhD, Shotai Kobayashi, MD, PhD, and Seung U. Kim, MD, PhD

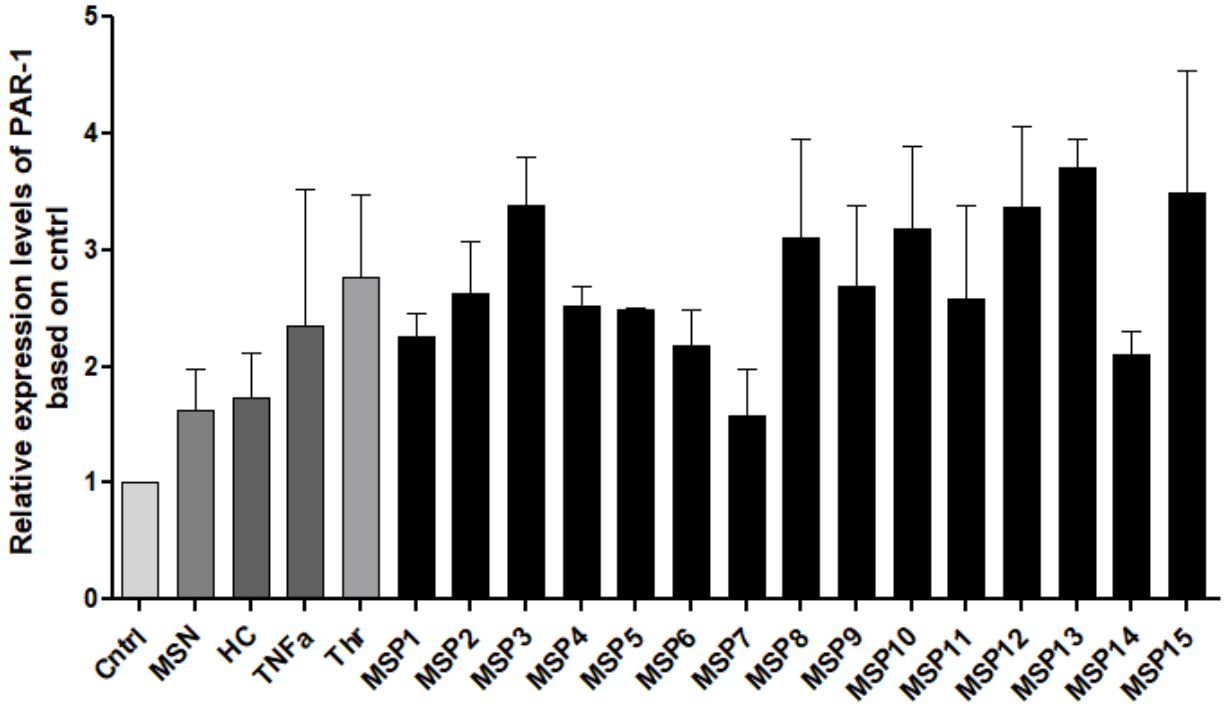
Higher relative expression of PAR-1 after 4 hours of exposure to stimuli compared to 1 hour (Ishida *et al.,* J. Neuropathol. Exp. Neurol. 2006).

In the current study

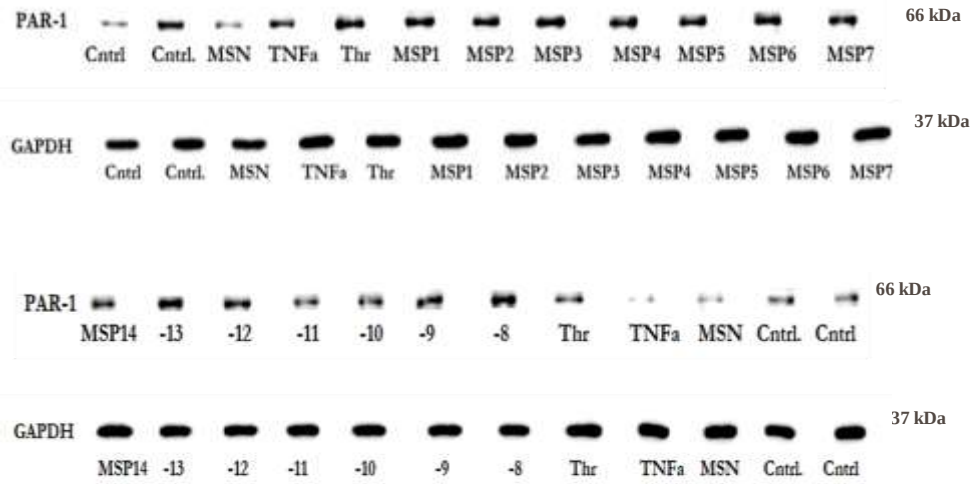
Four hours of activation with thrombin (5U/ml) or pooled sample containing antibodies of interest significantly affect PAR-1 expression levels.

Results

PAR-1 expression upon stimulation of U87 astrocytes with purified IgG antibodies against coagulation components



MSP: IgG fractions from positive MS patients; MSN: negative MS patients; HCs: healthy controls; TNF-a: positive control; Thrombin: positive control; Cntrl: unstimulated cells (negative control)

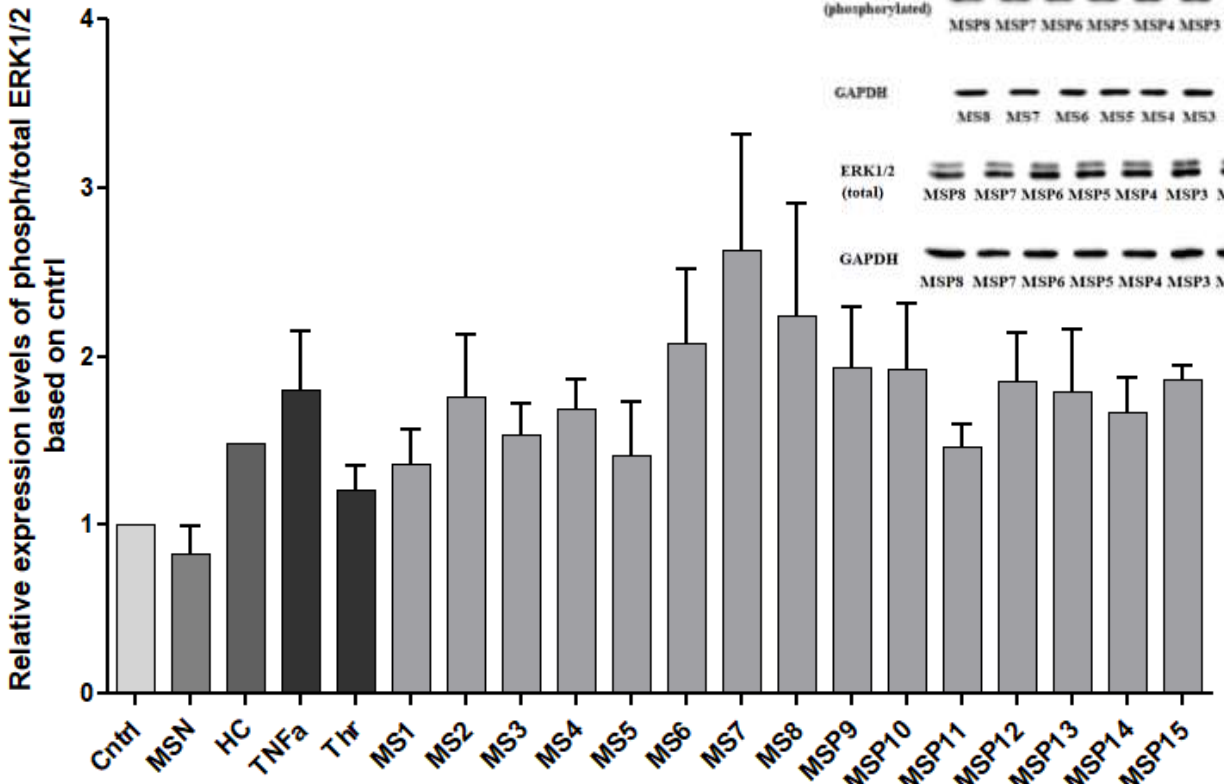


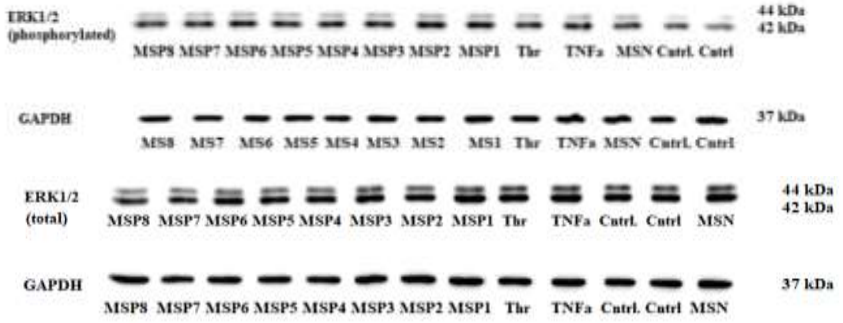
- Increased relative expression levels of PAR-1 upon astrocytic stimulation with purified IgG (MSP 1-15) compared to control.
- The highest relative expression levels observed after stimulation with anti-thrombin IgG antibodies (MSP-13).
- Astrocytes treated with samples derived from negative MS patients or HCs did not show any important fold changes

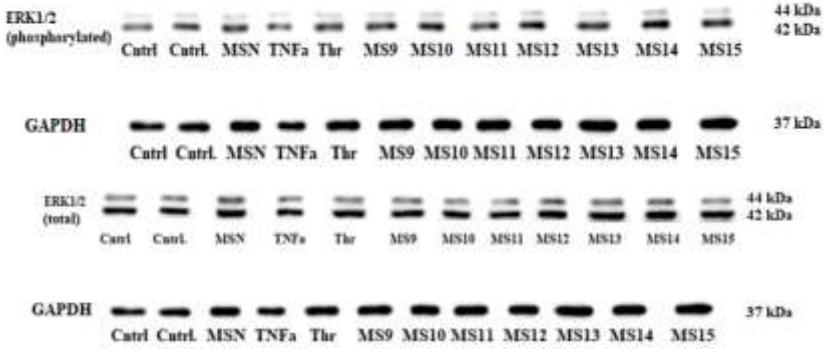
MSP samples	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Activity to	FXa	FVIIa	plasmin	Plasmin, FXa, FXII	Plasmin, FXa	Plasmin FXa	Plasmin, FXa, Thrombin	Plasmin, Prothrombin, FXII, Protein C	FXII	Protein C	Thrombin	Prothrombin	Thrombin	FVIIa	FXII

Results

Activated ERK1/2 expression upon stimulation of U87 astrocytes with purified IgG antibodies against coagulation components







- Up to 2.5-fold increased relative expression levels of activated ERK1/2 upon stimulation with purified IgG antibodies.
- The highest levels obtained following stimulation with samples with seropositivity for more than one antibodies (MSP6, MSP7, and MSP8).

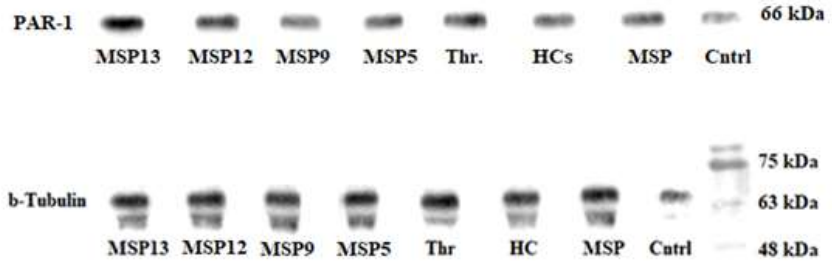
MSP: IgG fractions from positive MS patients; MSN: negative MS patients; HCs: healthy controls; TNF-a: positive control; Thrombin: positive control; Cntrl: unstimulated cells (negative control)

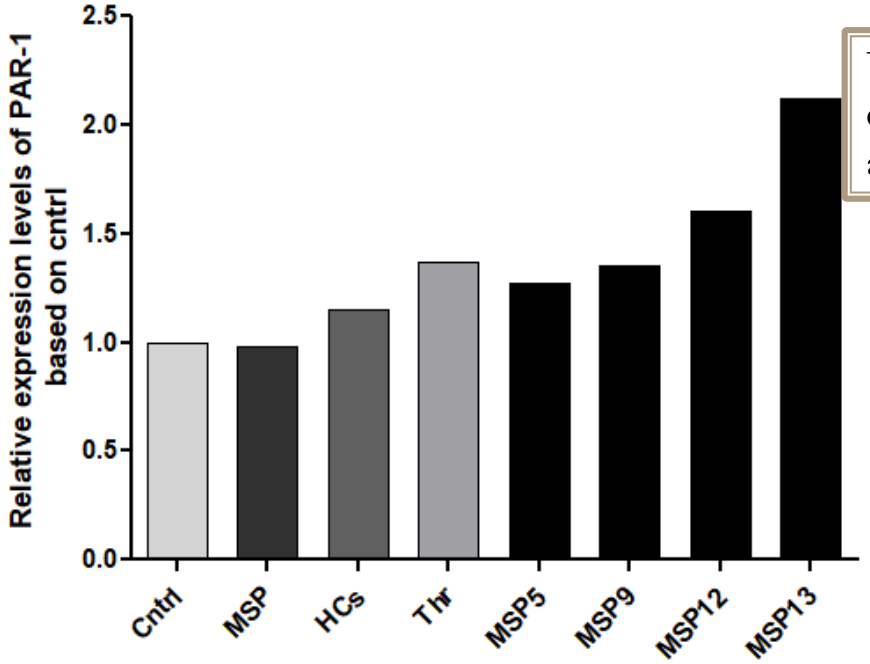
MSP samples	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Activity to	FXa	FVIIa	plasmin	Plasmin, FXa, FXII	Plasmin, FXa	Plasmin FXa	Plasmin, FXa, Thrombin	Plasmin, Prothrombin, FXII, Protein C	FXII	Protein C	Thrombin	Prothrombin	Thrombin	FVIIa	FXII

Results

Ex vivo analysis of the role of IgG antibodies against coagulant components

I. PAR-1 expression upon activation of primary human astrocytes

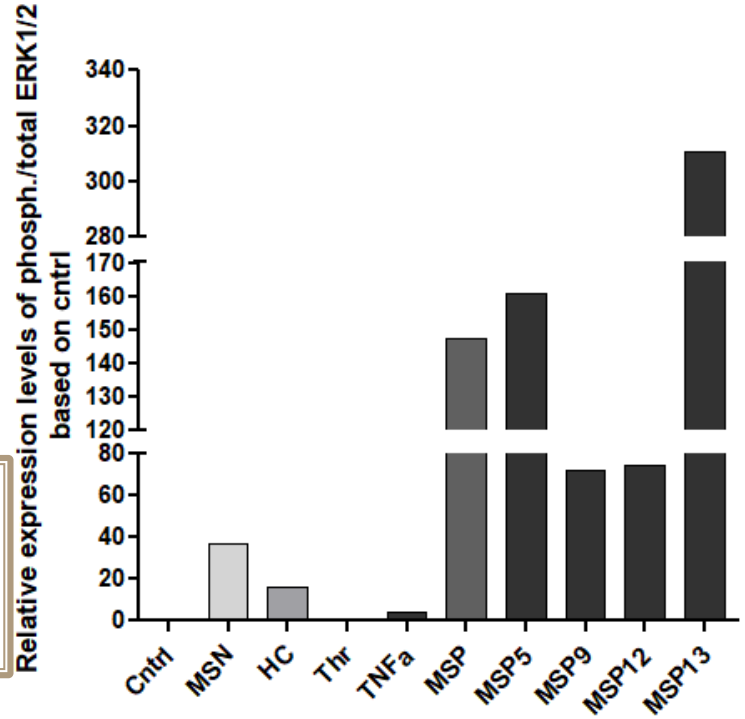




Upregulation of PAR-1, especially upon stimulation with anti-thrombin IgG (MSP-13).

Cntrl: unstimulated cells (negative control); MSP: Stimulated cells with IgG fractions from positive MS patients; HCs: stimulated cells with healthy controls samples; Thrombin: stimulated cells with thrombin (positive control)

II. Activation of ERK1/2 upon stimulation of primary human astrocytes



Upregulation of activated ERK1 and ERK2 kinases due to the presence of antibodies against coagulant components.

Cntrl: unstimulated cells (negative control); MSP: IgG fractions from positive MS patients; MSN: negative MS patients; HCs: healthy controls; TNF-a: positive inflammatory control; Thrombin: positive coagulant control

SUMMARIZING

IgG antibodies against coagulation components are associated with inflammatory diseases.

Our research has documented the presence of IgG antibodies against serine proteases of the coagulation cascade in a high proportion of patients diagnosed with Multiple Sclerosis.

The presence of antibodies - especially to thrombin - can promote the activation of pro-inflammatory pathways, contributing to disease progression.

The antibodies from MS patients can stimulate the expression of the thrombin PAR-1 receptor, a key player in thrombin's pro-inflammatory mechanisms, and are involved in pro-inflammatory pathways, such as activating ERK1/2 kinases.

IgG antibodies against coagulation antigens can be potential biomarkers in disease monitoring and prognosis, and they prove to be valuable tools for establishing new therapeutic strategies.