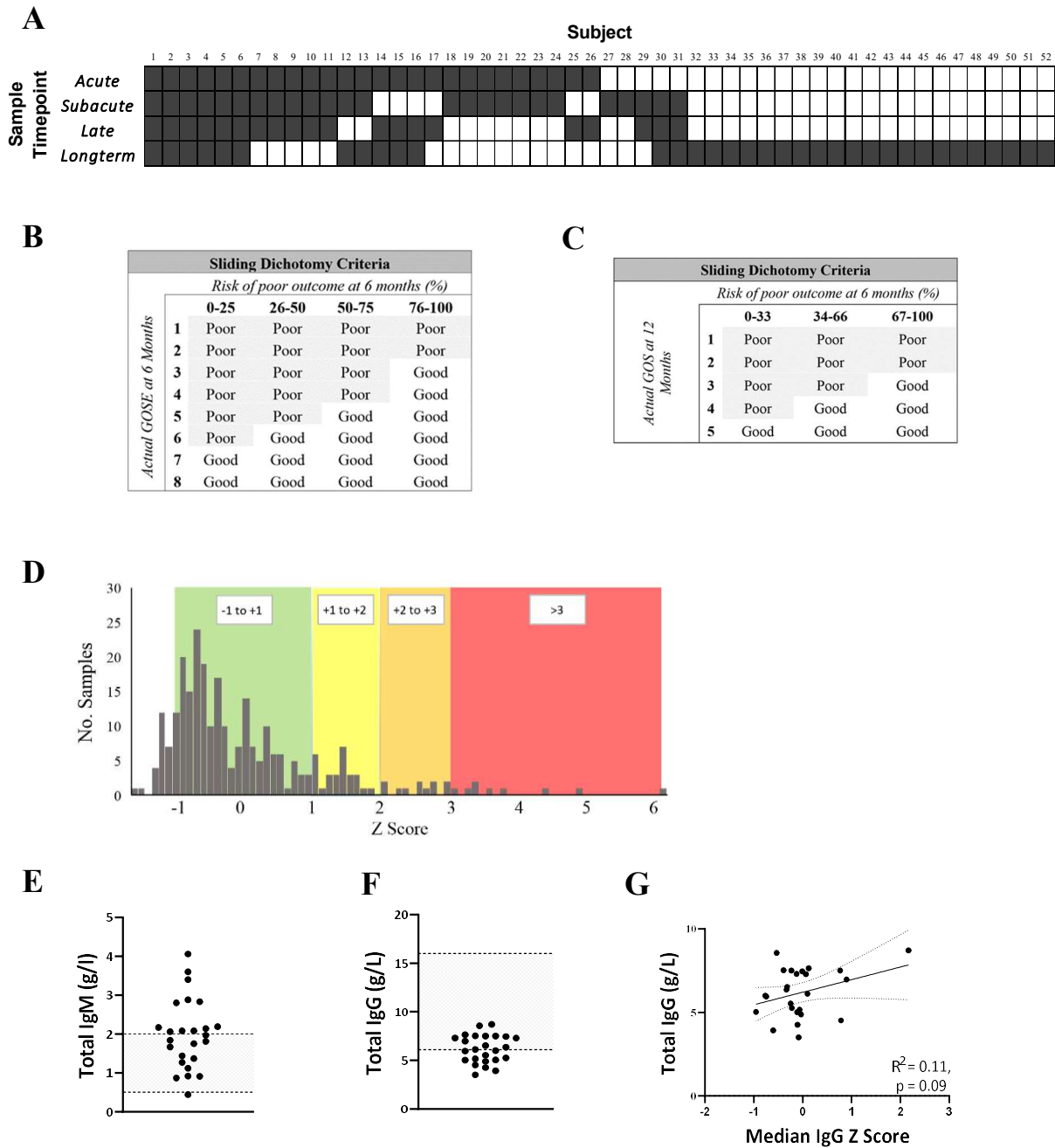
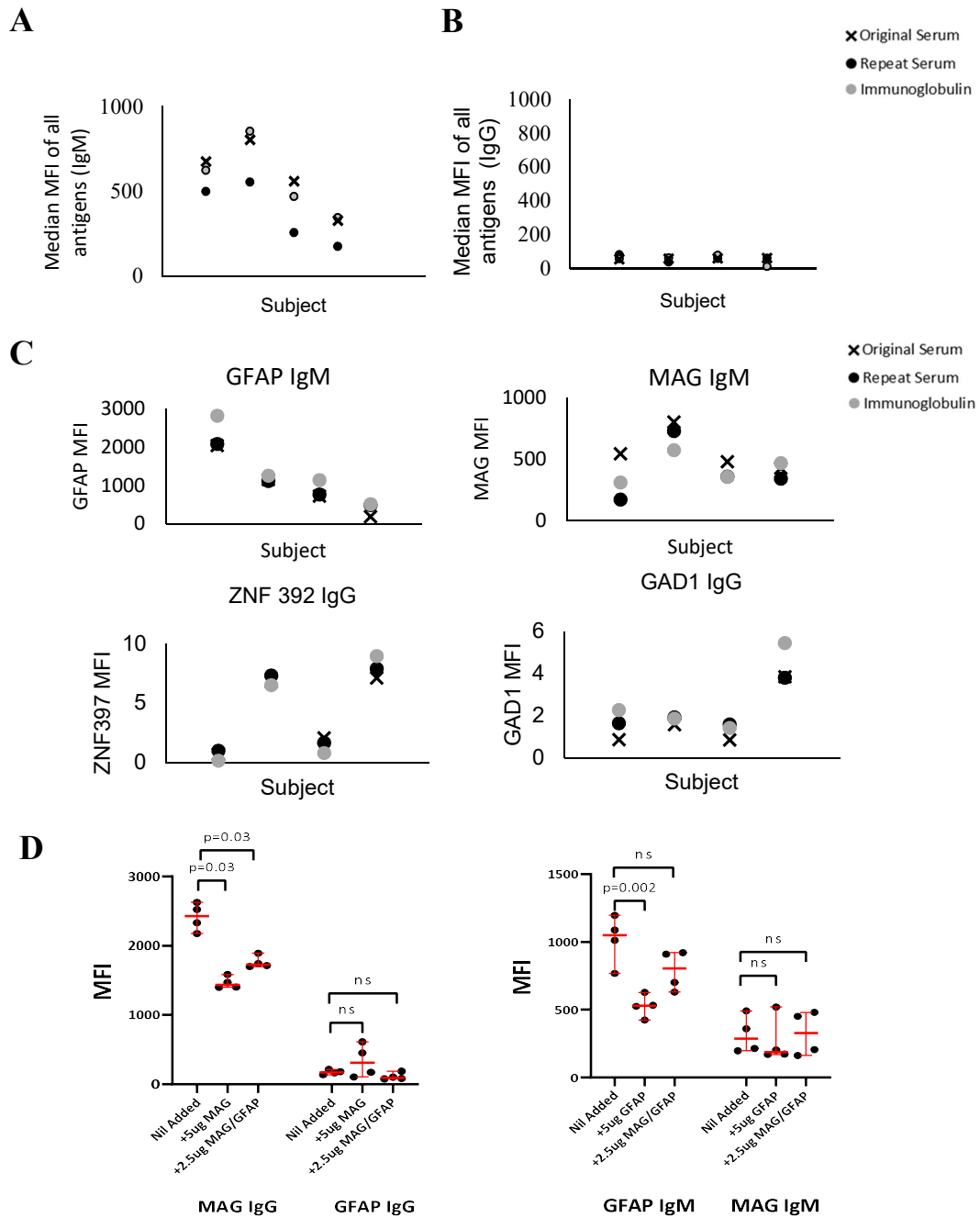


Supplementary Materials



Supplementary Figure 1. Schematic diagram displaying how patients from the Discovery cohort contributed to the Late and Long-term cohorts. *Shaded boxes represent time-points where samples were taken from individuals (A).* Criteria used to dichotomise patients' outcome into "worse than expected" and "as/better than expected" groups, according to their risk of poor outcome as judged by the IMPACT score variables for the Discovery cohort (B) and Validation cohort (C). Histogram displaying the distribution of Z scores of IgG MAG MFI in all samples used to generate the reference distributions (D). Total serum IgM concentrations was above the normal range in 12/25 patients. *Hatched area = normal range (E).* Total serum IgG concentration was below the normal range in 13/25 patients. *Hatched area = normal range (F).* Median IgG Z score did not correlate with total serum IgG (G).



Supplementary Figure 2. (A) Polyantigenic IgM response is replicated both in a technical repeat (serum assayed again in a separate experiment) and in the purified immunoglobulin fraction (B) no such replication is seen with IgG (C) Graphs displaying replication of dominant responses to specific antigens both in a technical repeat (serum assayed again in a separate experiment) and in the purified immunoglobulin fraction (D) To assess the antigen-specificity of the autoantibodies, two serum samples positive for anti-MAG IgG and anti-GFAP IgM respectively were pre-incubated with the corresponding cognate antigen. 200 ul serum was incubated for two hours at room temperature in the presence of 1) excess antigen (5 mcg of MAG or GFAP as appropriate), 2) 2.5 mcg of both MAG and GFAP to assess the effect of total protein added versus specific cognate antigen, and 3) with no antigen added. The samples were then diluted to 1 in 1000 and run as standard on the protein microarray. Maximal attenuation of the positive autoantibody signal was seen when serum was pre-incubated with the cognate antigen; no difference was seen in negative autoantibody signals.

Supplementary Material

Supplementary Table 1. Custom Protein Microarray Protein List					
Symbol	Protein Name	Location	Symbol	Protein Name	Location
Brain relevant (52)					
Aβ 42*	Amyloid Beta 42	EC/PM	PNMA1	Paraneoplastic Ma Antigen 1	IC
ANXA4	Annexin A4	PM	PNMA2	Paraneoplastic Ma Antigen 2	IC
APP	Amyloid Precursor Protein	EC/PM	S100B	S100 calcium-binding protein B	EC
AQP4*	Aquaporin-4	PM	SNCA	alpha-synuclein	IC
CDH13	Cadherin 13	EC/PM	SSB	Sjogren Syndrome Antigen B (La)	IC
CDR2	Cerebellar Degeneration Related Protein 2 (Yo; Purkinje)	IC	Tau*	Tau - 441	EC/PM
CHRNA10	Cholinergic Receptor Nicotinic Alpha 10 Subunit	PM	TPH1	Tryptophan Hydroxylase 1	IC
CHRNA9	Cholinergic Receptor Nicotinic Alpha 9 Subunit	PM	TROVE2	TROVE Domain Family Member 2 (Ssa/Ro)	IC
COL4A3BP	Collagen Type IV Alpha 3 Binding Protein	EC	TUBB3	Tubulin Beta 3	IC
DCN	Decorin	EC	ZIC4	Zinc finger protein	IC
DPYSL5	Dihydropyrimidinase-related protein 5 (CRMP5)	IC	BBB Relevant (5)		
DRD2	Dopamine receptor D2	PM	BSG	Basigin	PM
ELAVL4	(Embryonic Lethal, Abnormal Vision, Drosophila)-Like 4	IC	CLDN5	Claudin 5	PM
GABBR1	Gamma-Aminobutyric Acid Type B Receptor Subunit 1	PM	LAMC2	Laminin Subunit Gamma 2	PM
GABRA1	Gamma-Aminobutyric Acid Type A Receptor Alpha1 Subunit	PM	TJP1	Tight Junction Protein 1	PM
GABRB3	Gamma-Aminobutyric Acid Type A Receptor Beta3 Subunit	PM	SELE	E-Selectin	EC/PM
GAD1	Glutamate decarboxylase 1 (brain, 67kDa)	PM	Non-CNS / Control (22)		
GAD2	Glutamate decarboxylase 2 (65, kDa)	PM	ACE	Angiotensin Converting Enzyme	EC/PM
GFAP	Glial Fibrillary Acidic Protein	IC	BSA	Bovine Serum Albumin	Non-human
GLRA1	Glycine Receptor Alpha 1	PM	BSA-bio	Bovine Serum Albumin - biotinylated	Non-human
GRIA2	Glutamate Ionotropic Receptor AMPA Type Subunit 2	PM	CDH1	Cadherin 1	EC/PM
GRIA3	Glutamate Ionotropic Receptor AMPA Type Subunit 3	PM	CEACAM1	Carcinoembryonic Antigen Related Cell Adhesion Molecule 1	EC/PM
GRIA4	Glutamate Ionotropic Receptor AMPA Type Subunit 4	PM	CEACAM5	Carcinoembryonic Antigen Related Cell Adhesion Molecule 5	EC/PM
GRIN1	Glutamate Ionotropic Receptor NMDA Type Subunit 1	PM	CENPB	Centromere protein B	IC
GRIN2A	Glutamate Ionotropic Receptor NMDA Type Subunit 2A	PM	CENPH	Centromere Protein H	IC
GRIN3A	Glutamate Ionotropic Receptor NMDA Type Subunit 3A	PM	COL1A1	Collagen, type I, alpha 1	EC
GRIN3B	Glutamate Ionotropic Receptor NMDA Type Subunit 3B	PM	COL5A2	Collagen Type V Alpha 2 Chain	EC
GRINA	Glutamate Ionotropic Receptor NMDA Type Subunit Associated Protein 1	PM	DBT	Dihydroliipoamide Branched Chain Transacylase E2	IC
GRM1	Glutamate Metabotropic Receptor 1	PM	DDC	Dopa Decarboxylase	EC
GRM2	Glutamate Metabotropic Receptor 2	PM	DLAT	Dihydroliipoamide S-Acetyltransferase	IC
GRM3	Glutamate Metabotropic Receptor 3	PM	IFNA1	Interferon Alpha 1	EC
GRM4	Glutamate Metabotropic Receptor 4	PM	KRT18	Keratin 18	EC
GRM7	Glutamate Metabotropic Receptor 7	PM	MPO	Myeloperoxidase	EC
GRM8	Glutamate Metabotropic Receptor 8	PM	NUP210	Nucleoporin 210	IC
KCNJ10	Potassium Voltage-Gated Channel Subfamily J Member 10 (Kir4.1)	PM	PRTN3	Proteinase 3	EC/PM
LG11	Leucine-rich glioma inactivated 1	EC	TGM2	Tissue Transglutaminase	EC/PM
MAG	Myelin Associated Glycoprotein	PM	TPO	Thyroid Peroxidase	EC/PM
MBP	Myelin Basic Protein	PM	TSHR	Thyroid Stimulating Hormone Receptor	PM
MOG	Myelin Oligodendrocyte Glycoprotein	PM	ZNF397	Zinc Finger Protein 397	IC
NEFL	Neurofilament Light	IC			
NOVA1	RNA-binding protein Nova-1 (Ri)	IC			
OMG	Oligodendrocyte Myelin Glycoprotein	PM			

Supplementary Table 1. Full list of antigens printed on custom central nervous system protein microarray. *Location* relates to dominant subcellular location of expression derived from UniProtKB database. * relates to proteins that were sourced externally from the HuProt library EC = extracellular, PM = plasma membrane, IC = intracellular

Supplementary Material

Supplementary Table 2. Statistics for All Group Comparisons									
Discovery	<i>Acute</i>			<i>Subacute</i>					
Polyantigenic IgM	-0.06	[-0.2- 0.13]	1.19	[0.7-1.80]	p<0.0001				
Polyantigenic IgG	-0.34	[-0.47—0.15]	-0.26	[-0.42--0.09]	p=0.035				
Validation	<i>Acute</i>			<i>Subacute</i>					
Polyantigenic IgM	0.14	[-0.44-0.28]	0.46	[-0.14-1.35]	p<0.001				
Polyantigenic IgG	-0.18	[-0.50-0.26]	0.03	[-0.37-0.50]	p=0.003				
Discovery	<i>Worse than Expected</i>			<i>As / Better than Expected</i>					
Polyantigenic IgM	2.74	[1.89-5.64]	1.46	[0.41-2.72]	p=0.01				
Ag Dominant IgM	1.5	[1-3]	2	[0.5-5]	p=0.43				
Ag Dominant IgG	1.5	[0-4.5]	1	[0-6.5]	p=0.70				
Validation	<i>Worse than Expected</i>			<i>As / Better than Expected</i>					
Polyantigenic IgM	0.62	[-0.15-1.46]	0.21	[-0.36-1.17]	p=0.15				
Ag Dominant IgM	1	[0-2]	1	[1-2]	p=0.67				
Ag Dominant IgG	1	[0-2]	1	[1-2.25]	p=0.33				
Col5a2 IgG	<i>Lung Contusions</i>			<i>No Lung Contusions</i>					
IgG	1.6	[1.14-2.02]	1.11	[1.00-1.17]	p=0.04				
Late	<i>Late</i>			<i>Healthy Controls</i>					
NfL(pg/ml)	31.1	[17.9-61.0]	6.8	[5.6-10.7]	p<0.0001				
GFAP (pg/ml)	73.6	[52.3-123.2]	55	[38.3-79.4]	p=0.05				
	<i>Late</i>			<i>Day 0 Post-TBI</i>			<i>Healthy Controls</i>		
Polyantigenic IgM	0.54	[0.27-0.95]	0.28	[0-0.52]	p=0.0002	-0.27	[-0.56-0.45]	p<0.0001	
Polyantigenic IgG	-0.14	[-0.27—0.01]	-0.33	[-0.45- -0.16]	p<0.0001	-0.44	[-0.53--0.32]	p<0.0001	
Long-term	<i>Long-term</i>			<i>Healthy Controls</i>					
NfL (pg/ml)	15.8	[8.24-23.8]	14.4	[10.3-17.9]	p=0.4				
GFAP (pg/ml)	145	[110-250]	118	[97-181]	p=0.11				
Polyantigenic IgM	-0.23	[-0.21- -0.31]	-0.31	[-0.38- -0.3]	p=0.0002				
Polyantigenic IgG	-0.18	[-0.31- -0.11]	-0.37	[-0.45- -0.28]	p<0.0001				
MAG IgG	-0.35	[-0.69-0.17]	-0.24	[-0.61-0.22]	p=0.85				
SELE IgG	-0.12	[-0.49-0.36]	-0.23	[-0.53-0.46]	p=0.83				
MAG IgM	-0.26	[-0.58-0.25]	-0.24	[-0.53-0.35]	p=0.76				

Supplementary Table 2. Values for statistics quoted in body of main text. “Polyantigenic” values refer to the median Z score per patient, “Ag (antigen) dominant” values refers to the number of dominant antibodies against specific antigens detected per patient. MAG/SELE values refer to the Z scores of the particular antigen-specific autoantibody. All values represent Median [IQR].

