

Supplementary Figures and Tables

Cytometric bead array kit assay

The CBA Th1/Th2/Th17 assay (BD Bio-sciences, California, USA) kit was used to analyse the concentration of interleukin (IL-2), IL-4, IL-6, IL-10, tumor necrosis factor (TNF), interferon-gamma (IFN- γ), and IL-17A cytokines from 31 AP patients (15 MAP, 11 MSAP and 5 SAP) as well as 6 controls on days 1, 3, 5 and 7 post epigastric pain. This was performed according to the manufacturer's instructions. The kit consists of antibody-coated beads, which are used to bind to cytokines present in the samples. A standard assay provided by the manufacturer was used for acquiring 10,000 events per sample on an LSRFortessa™ II flow cytometer (BD Biosciences USA) for the experiment. The concentrations were all determined from standard curves (concentration of standards were between 0.00 and 5,000pg/mL). In most cases, the extrapolated concentration (fitted CC) was below zero and the Mean fluorescence intensity (MFI) was used for data analysis.

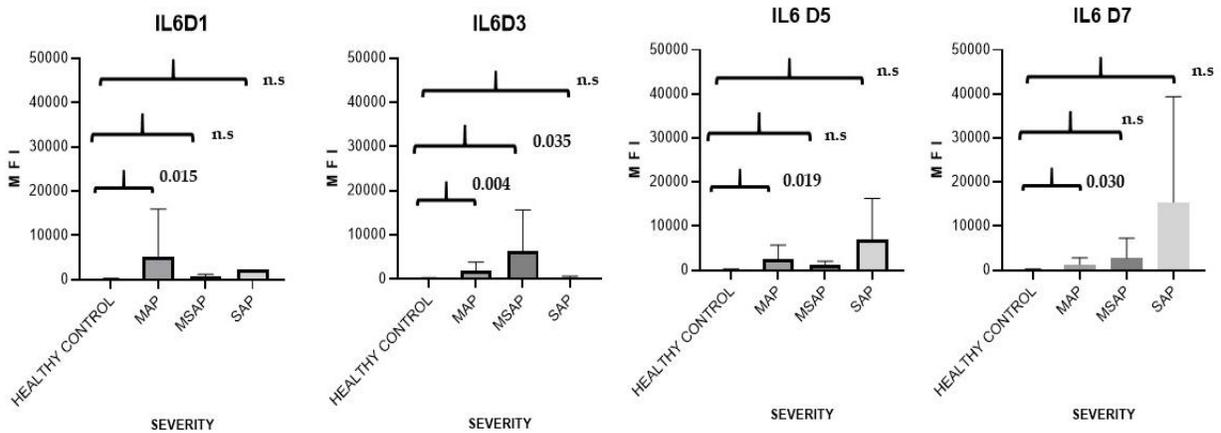


Figure S1 Protein analysis using the Th1/Th2/Th17 cytometric bead array (CBA) kit In the MAP and MSAP group, IL-6 showed significant differences on Day 3 compared to the healthy control group with $P=0.004$ and $P=0.035$ respectively. On Day 5 the MAP showed significance with $P= 0.019$ and the MSAP group had a significant difference with $P=0.030$ compared to the healthy control group. D: Day e.g. Day1, 3, 5, 7 of epigastric pain; MFI: median fluorescent intensity; IL: interleukin; MAP: mild acute pancreatitis; MSAP: moderately severe acute pancreatitis; SAP: severe acute pancreatitis.

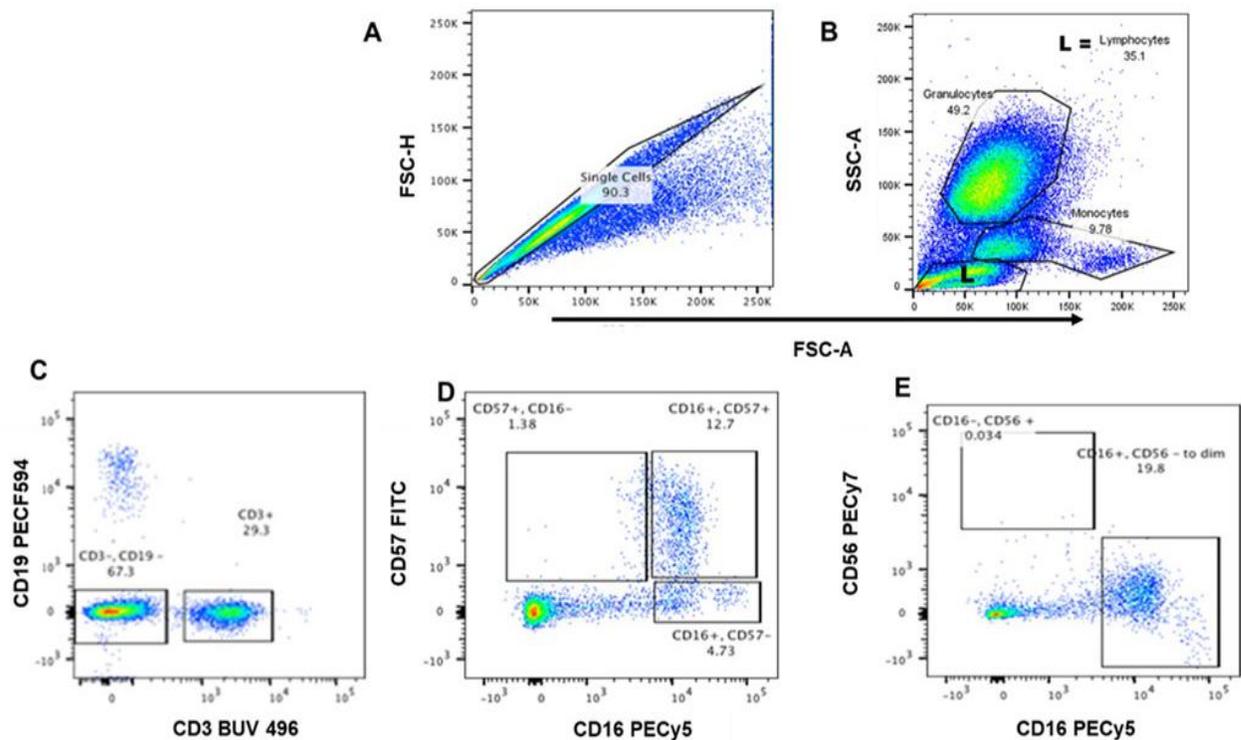


Figure S2 A plot of lymphocytes for an MSAP patient on Day 3 after AP post epigastric onset. A: Shows a FSC-H vs FSC-A to discriminate doublets from singlets. B shows a plot of SSC-A vs FSC-A to discriminate lymphocytes, monocytes, and granulocytes. C: B cells (PECF594 CD19+) were discriminated from BUV496 CD3+ cells (Alexa fluor CD4 and BV605 CD8 T cells and BUV496 CD3- cells, which include NK cells and other groups of ILCs). D and E: show NK cell subsets, which are CD16+/- CD57+/- (PECy7CD16, CD57FITC) and CD16+/-CD56+/- (CD16 PECy5, CD56PECy5) cell subsets respectively. The CD3-CD16 +CD57+ cells were 12.7 percent and CD3-CD16+CD57- cells were 4.7%. The CD3-CD16+CD56- cell subsets were 19.8% and the CD3-CD16+CD56+ were less than 0.1 %. FSC-H: forward scatter height; FSC-A: forward scatter area; BUV: BD Horizon Brilliant™ Ultraviolet; Cy: Cyanine; BV is Brilliant Violet™; PE: Phycoerythrin; PerCP: Peridinin-Chlorophyll-protein; CD: cluster of differentiation.

Table S1 Characteristics of Innate Lymphoid Cells [22, 52]

	ILC1s (including NK cells)	ILC2s	ILC3s
Transcription factors	T-bet	GATA3, ROR α	ROR γ t
Cytokines required for differentiation into ILC group	IL-15, TGF- β , GM-CSF	IL-25, IL-33, TSLP	IL-23
Cytokine produced by ILC group	IFN- γ , TNF- α , IL-22, VEGF, CXCL8	IL-5, IL-13, IL-6, IL-9	IL-22, IL-17, GM-CSF, IFN- γ

IL: interleukin; *T-bet*: T-box transcription factor; *TGF- β* : Tumor growth factor-beta; *IFN- γ* : interferon-gamma; *TNF- α* : tumor necrosis-alpha; *VEGF*: Vascular endothelial growth factor; *CXCL8*: chemokine receptor 8; *GATA-3*: G-A-T-A 3 transcription factor; *ROR α* : RAR Related Orphan Receptor alpha; *TSLP*: thymic stromal lymphopoietin; *ROR γ t*: RAR Related Orphan Receptor gamma t; *GM-CSF*: granulocyte-macrophage colony-stimulating factor; ILC: lymphoid cells, group; NK: natural killer.

Table S2 Optimised multicolour flow cytometry panel used for analysis and characterization of white blood cells in AP patients

Filter	Parameter	B cells	Granulocytes	T Cells	NK Cells
780/60 BP	APC-Cy7		CD11b		
730/45 BP	Alexa Fluor 700			CD4	
660/20 BP	APC			CD45RO	
780/60 BP	PE-Cy7				CD56
695/40	PerCP-Cy5-5		CD14		
660/20	PE-Cy5				CD16
610/20 BP	PE-CF594	CD19			
530/30BP	FITC			CD57	
655/8	BV650			HLA-DR	
605/12	BV605			CD8	
450/50 BP	BV421			CCR7/CD197	
530/30	BUV496			CD3	

APC: Allophycocyanin; BUV: BD Horizon Brilliant™ Ultraviolet; BP: bandpass Cy: Cyanine; CCR7: chemokine receptor type 7; FITC: Fluorescein isothiocyanate; BV is Brilliant Violet™; HLA DR: human leukocyte D related; PE: Phycoerythrin; PerCP: Peridinin-Chlorophyll-protein; CD: cluster of differentiation; CCR: C-C chemokine receptor type. All antibodies are from BD Biosciences, (New Jersey, USA).

Table S3 List of genes that were upregulated and downregulated in AP patients. *CCR8* was the most upregulated gene in severe AP with a fold regulation of 1172.45.

GENE SYMBOL	MILD(MAP)	MODERATE (MSAP)	SEVERE (SAP)
<i>APCS</i>	1.33	262.91	-1.01
<i>CASP1</i>	-1.43	3.02	3.38
<i>CCR8</i>	1.33	38.28	1172.45
<i>IL10</i>	-1.30	58.62	-1.47
<i>IL13</i>	-1.92	83.66	19.53
<i>IL17A</i>	1.72	116.93	2.56
<i>IL23A</i>	-5.60	18.07	6.57
<i>IL4</i>	-1.13	108.64	36.83
<i>IL5</i>	1.33	192.59	1.21
<i>NOD1</i>	-8.93	-14.62	64.21
<i>MPO</i>	1.33	91.77	6.82

***APCS*: Amyloid P component serum; *CASP1*: Caspase 1; apoptosis-related cysteine peptidase; *CCR8*: Chemokine receptor 8; *IL*; (interleukin) 4; 5; 10; 13 17A; 23A; 10; *NOD1*: Nucleotide-binding oligomerization domain-containing protein 1; *MPO*: myeloperoxidase.**