Online supplemental material

Supplemental methods:

Protein analysis and IHC. Isolation of acinar cells and immunoblot analysis were performed as described (21). Paraffin sections (3 μm) were stained using standard IHC procedures.

Antibodies and Hyper-IL-6. Immunoblot analysis and IHC were performed using antibodies to the following: STAT3^{Y705} (sc-482, Santa Cruz), STAT3 (610189, BD Pharmingen), p-STAT3^{Y705} (9131 for western blot and 9145 for IHC, Cell Signaling), p-STAT3^{S727} (9134, Cell Signaling), IκBα (sc-371, Santa Cruz), IκBβ (sc-945, Santa Cruz), RELA (3034, Cell Signaling), ERK 1/2 (sc-93, sc-154, Santa Cruz), p-IκBα (9246, Cell Signaling), IL-6 (ab6672, Abcam), SOCS3 (sc-7010, Santa Cruz), p-p38 (4631, Cell Signaling), p-MYPT1 (3048, Cell Signaling), p-MLC (3674, Cell Signaling), p-STAT1^{Y701} (9171, Cell Signaling), p-RELA (3033, Cell Signaling), LAMIN A/C (sc-20681, Santa Cruz), and β-ACTIN (A 5441, Sigma). Purified Hyper-IL-6 has been described in a previous study (27).

Preparation of serum and tissue samples. Mice were sacrificed at the indicated time points. Serum, pancreases, lungs, and livers were harvested. Lipase and amylase were measured using standard procedures. MPO activity (i.e., the quantification of pulmonary neutrophil infiltration) in lung samples was measured via an established protocol (21).

Blood pressure. Blood pressure was noninvasively measured before the first injection of cerulein on days 1, 2, and 4 (n=4) by determining the tail blood volume with a volume pressure-recording sensor and an occlusion tail-cuff (CODA System, Kent Scientific, Torrington, CT).

Endotoxin level. Endotoxin levels were analyzed in the serum of C57BL/6 mice at different time points according to the protocol of the manufacturer (Limulus amebocyte lysate [LAL] QCL-1000, Lonza).

β-Galactosidase staining. Cells, tissues, and embryos were fixed in 4% paraformaldehyde at 4°C and washed three times in lacZ wash buffer (i.e., 2 mM $MgCl_2$, 0.01% sodium deoxycholate, 0.02%

Nonidet P-40 in PBS) for 30 min. β-Galactosidase activity was detected as previously described. Stereoscopic pictures were taken using a Zeiss Stemi 11 microscope. For the histological analysis, the harvested organs were cryosectioned after fixation, post-fixed in 0.2% glutaraldehyde in PBS, and stained as described above. Counterstaining was done with eosin or nuclear fast red.

RNA analysis. Total tissue RNA was extracted using the RNeasy kit (Qiagen) according to the manufacturer's instructions as previously described (37). Reverse-transcriptase (i.e., Invitrogen) was used for the synthesis of cDNA. Transcribed cDNA was further analyzed via real-time PCR using the Power SYBR Green PCR Master Mix (Applied Biosystems). All values were normalized to the level of Cyclophilin cDNA. The primer sequences can be found in the table below.

Name	Primer forward (5`-3`)	Primer reverse (5`-3`)
Cyclophilin	ATGGTCAACCCCACCGTGT	TTCTGCTGTCTTTGGAACTTTGTC
IL-6	GAAGTAGGGAAGGCCGTGG	CTCTGCAAGAGACTTCCATCCAGT
Cxcl1	GGCGCCTATCGCCAAG	CTGGATGTTCTTGAGGTGAATCC

Supplemental figure legends

Supplemental Figure 1: (**A)** and (**B**) Serum was removed for amylase and lipase analysis during AP at indicated time points. (**C**) The endotoxin activities of different serum samples were determined with a LAL assay at indicated time points. (**D)** and (**E**) Blood pressure was analyzed in mice during SAP. (**F-H**) Serum was removed for ALT, AST, and BUN analysis at indicated time points. (**I)** and (**J**) Representative H&E sections of liver and kidney tissue of C57BL/6 mice during SAP at indicated time points. Necrotic cells were found after 3 days of AP in the liver tissue (dashed line). Scale bars represent 50µm. Results represent mean ± SD (n >4), *p < 0.05, **p < 0,005, ***p < 0,001 versus 0 hours.

Supplemental Figure 2: (**A**) IHC stainings of pIκBα (upper panel) and p-p38 (lower panel) in the lung tissue of C57BL/6 mice during SAP. (**B**) At 0, 4, and 8 hours, pancreatic tissue was isolated and homogenized to detect p-MLC and p-MYPT1. Erk1/2 served as the loading control (representative blot; n=4 for each time point).

Supplemental Figure 3: (**A**) Isolated acinar cells were incubated with a supramaximal concentration of cerulein for different time points. Protein lysates from incubated acinar cells were homogenized and blotted with p-STAT3^{Y705} and STAT3. β-actin served as the loading control (representative blot; n=3). (**B**) Serum sIL-6R level in C57BL/6 mice during SAP (n=4). (**C**) BALF sIL-6R level in C57BL/6 mice during SAP (n=4). Results represent mean ± SD; **p < 0.005.

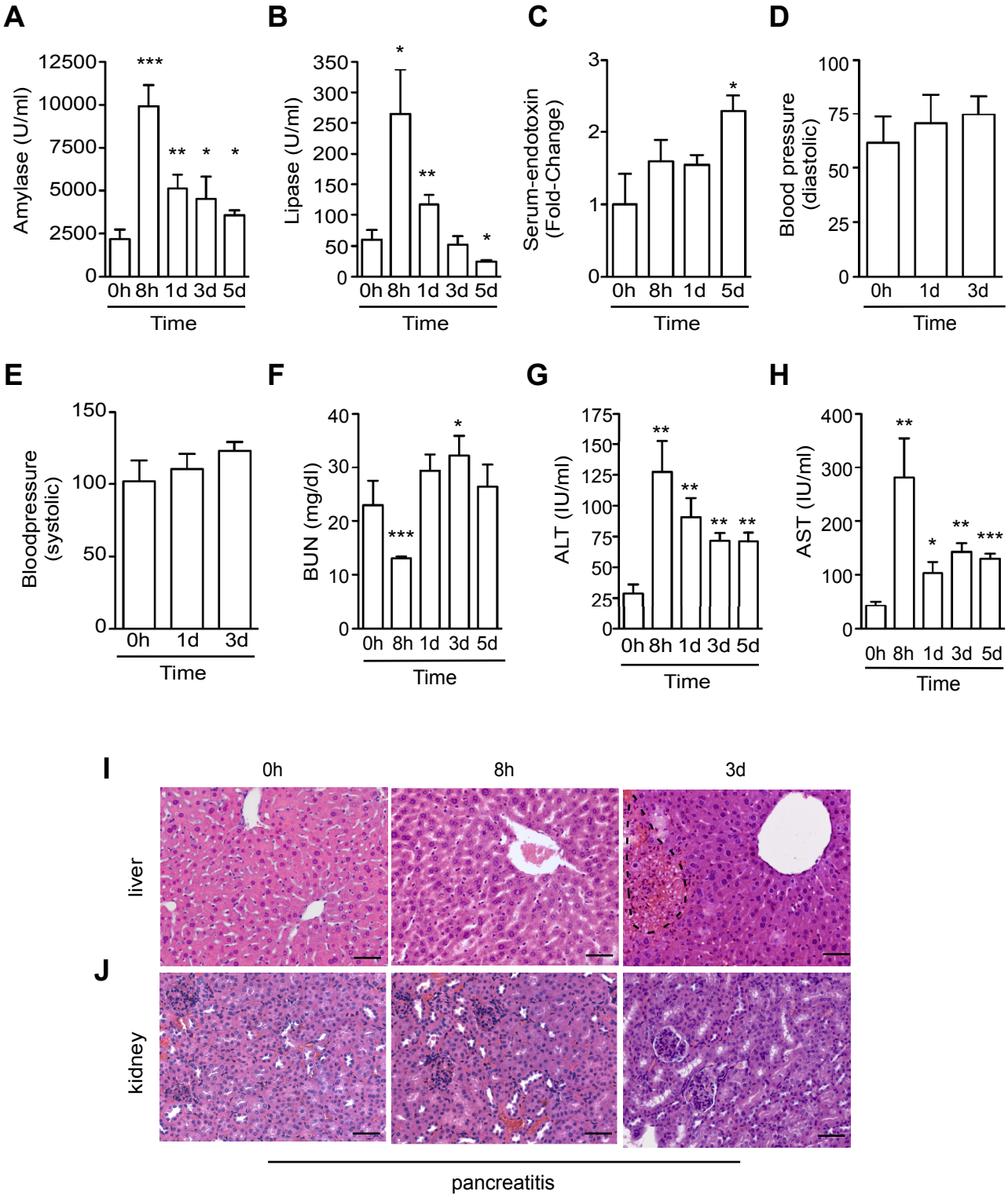
Supplemental Figure 4: (**A**) Immunoblot analysis of pancreatic acinar cells using different STAT3 antibodies. STAT3^{Y705} (C-20, Santa Cruz) is directed against the tyrosine 705-containing domain in the C-terminus, while the second antibody (#610190BD; Pharmingen) detects the N-terminus (amino acid 1-175) of truncated STAT3$^{\Delta panc}$ and wild-type STAT3 (representative blot; n=3 mice). (**B**) Expression of SOCS3 during AP. IHC analyses was used to localize SOCS3 in mice undergoing AP. After 8 hours of expression of SOCS3 was detectable in acinar cells of *Socs3*$^{F/F}$ mice, while the acinar cells of *Socs3*$^{\Delta panc}$ mice were negative for the expression of SOCS3. (**C**) Serum was removed for lipase analysis at the indicated time points. Note the increased release of lipase into the serum of *Socs3*$^{\Delta panc}$ mice, while levels remained lower in *Stat3*$^{\Delta panc}$ mice as compared to floxed littermate controls (*Stat3*$^{F/F}$

and *Socs3*$^{F/F}$). (**D**) Pancreatic edema increased as measured by relative pancreatic weight (PW= pancreatic weight, BW= body weight; n=8). Values are mean ± SD for independent animals (n = 8). *p < 0.05 and **p < 0.005 versus *Stat3*$^{F/F}$.

Supplemental Figure 5: (**A**) Schematic of treatment of SAP. Mice were injected IP twice with the recombinant soluble gp130Fc (150µg), the CXCR2 antagonist SB225002, and an anti-CXCL1 antibody, or mice were injected 5 times IV with the small-molecule STAT3 inhibitor S3I-201 (7.5 µg/g body weight). (**B**) IHC staining of p-STAT3^{Y705} in the pancreatic tissue of C57BL/6 mice treated with DMSO or S3I-201. Note the missing p-STAT3 translocation in S3I-201 treated mice. Scale bars represent 50 µm.

Supplemental Table 1: Characteristics of the Patients

Supplemental figure 1



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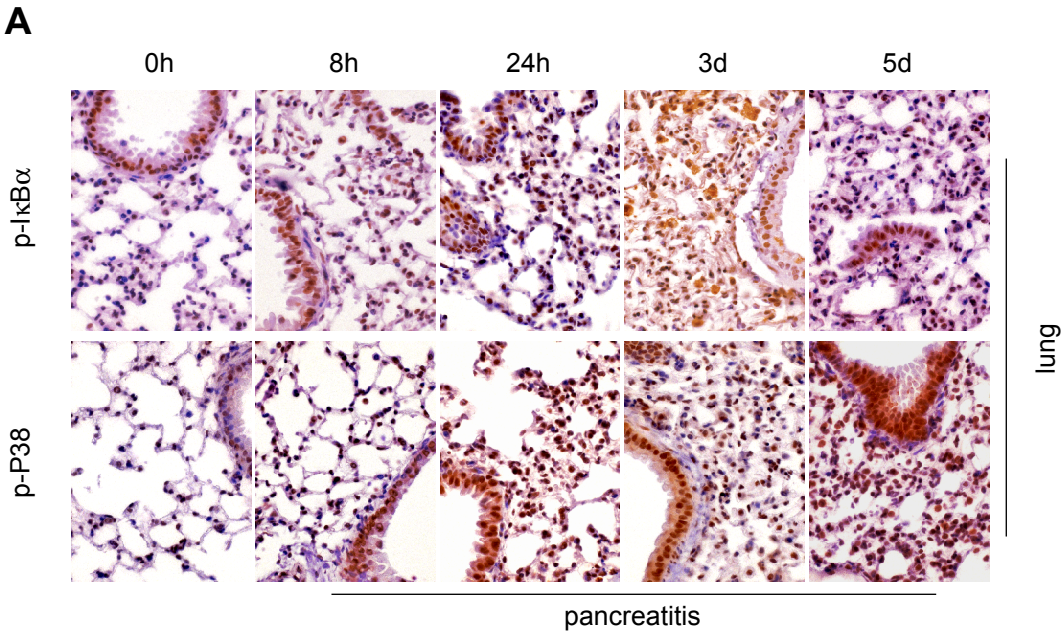

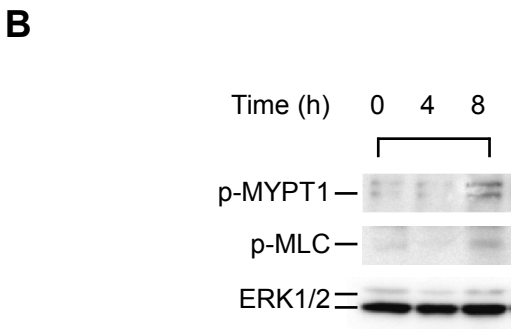

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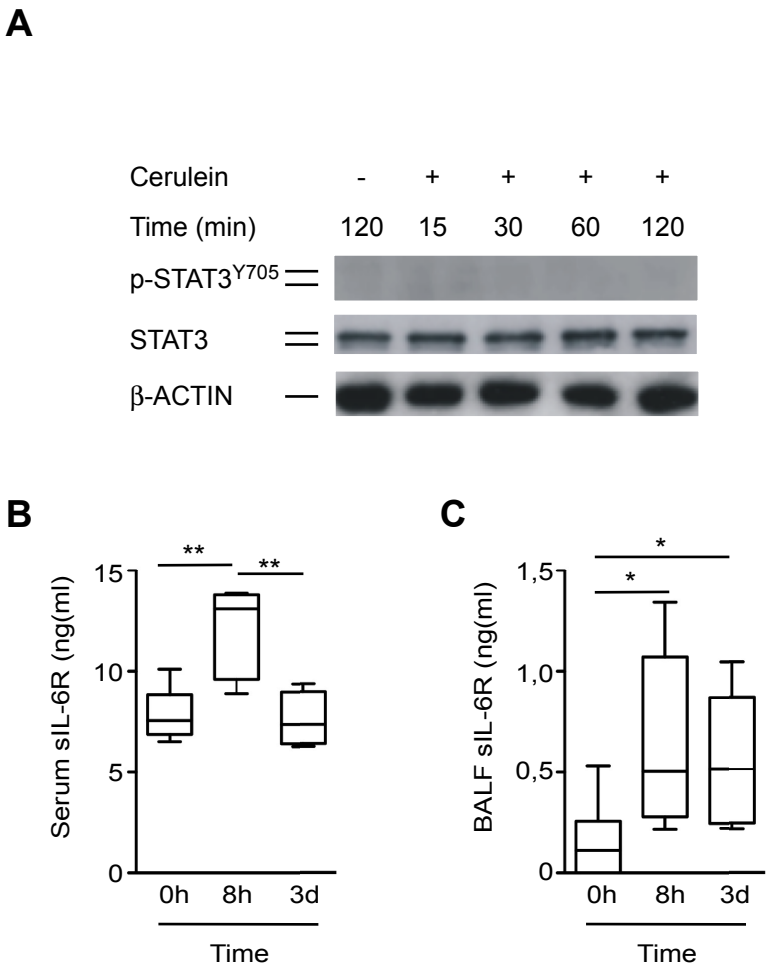

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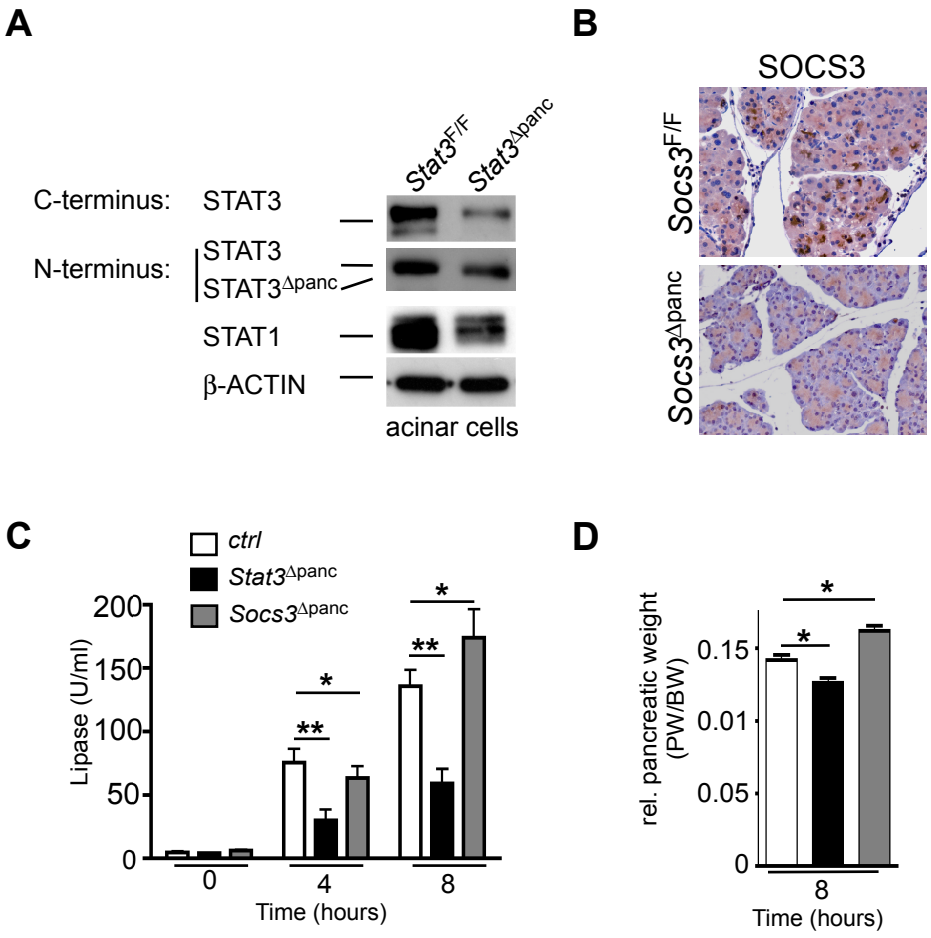

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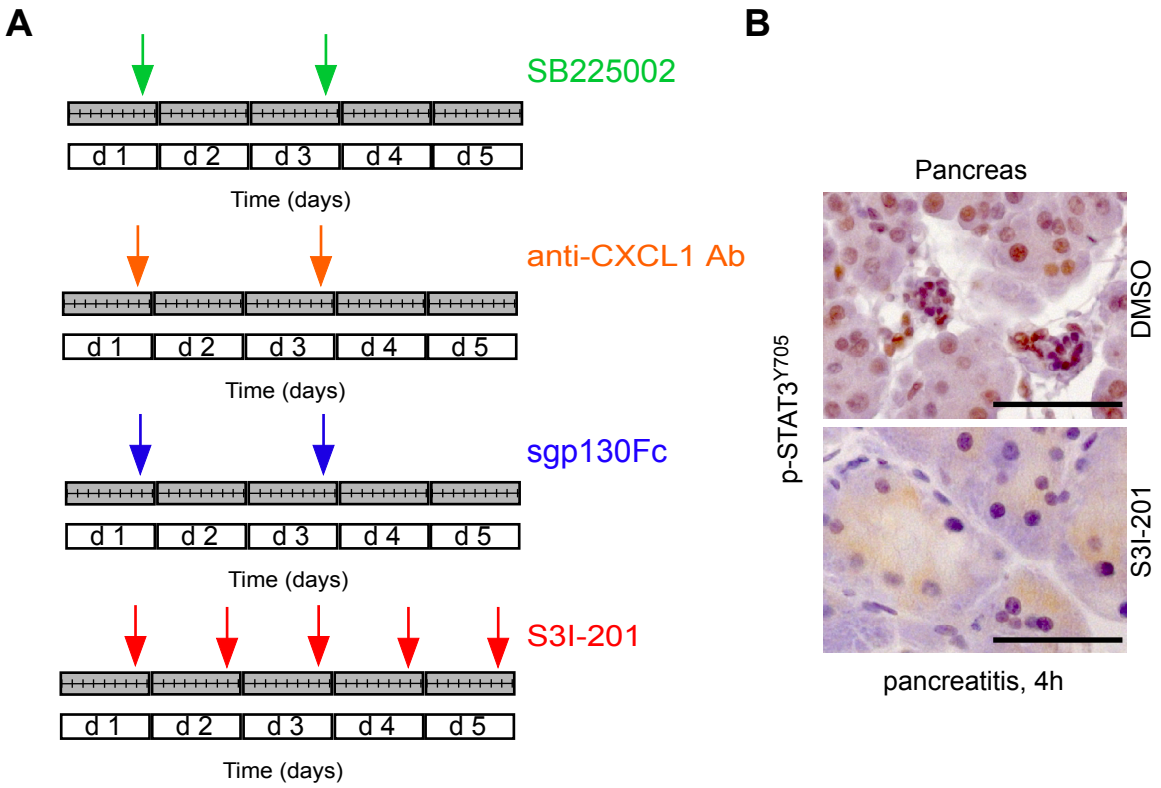

Supplemental Table 1: Patients with acute pancreatitis

Patient ID	Gender	Age	Severity Atlanta	Etiology	MOF		Chest X-ray
					pulmunary	other	
004:2	m	56	severe	5	No ventilation; Oxygenation 89% with 2,5 L oxygen and pleural effusions led to pleural drainage. Lowest oxygenation value 86% without oxygenation and 90% with 4L oxygen.	Pancreatic abscesses, treated with drainage	
077:2	m	89	severe	2	Pulmonary edema; Lowest oxygenation value 79% without oxygenation and 74% with 2L oxygen (at this time dyspnea is noted).	Cardiac failure, death	
163:2	m	92	severe	0		Cardiac failure	
200:3	m	31	severe	1	Mechanical ventilation; PaO2/FiO2 levels ranging between 18 and 35 over > 3months ICU care.	Renal failure; pancreatic abscesses	Pleural effusions and parenchyma changes
227:2	f	57	severe	0	Arterial pO2 level at lowest 7,8 ranging to normal over 3 days of ICU care	ICU care for circulatory and cardiac reasons (hypo and hypertoni and tackycardia, various pharmacologic treatments). EDA for pain.	
318:3	m	77	severe	0		Pseudocyst	
553	f	81	severe	0	Mechanical ventilation (death after 5 days) PaO2/FiO2 levels ranging between 12 and 55 over the 5 days of mechanical ventilation. After that NIV a couple of times for 2 days.	Renal failure	Pleural effusions
611	f	62	severe	2	Mechanical ventilation for 5 days. PaO2/FiO2 levels ranging between 19 and 56 over 5 days of mechanical ventilation. After that intermittent NIV for 2 days.		Pleural effusions, drained
618	m	81	severe	5	NIV at ICU for 4 days. Pao2/FiO2 between 17 and 70 during these days.	Circulatory hypotonia. NA treatment. Abdominal abscess	Pleural effusions
M1	f		severe	2	ARDS, mechanical ventilation for 10 days (death after 10 days)	Abdominal compartment syndrome	Bilateral diffuse, pulmonar infiltrate

021:3	f	57	mild	0	no	no	no pathology
024:2	m	74	mild	0	no	no	no pathology
026:2	f	47	mild	0	no	no	no pathology
036:2	f	55	mild	5	no	no	no pathology
059:2	f	78	mild	4	no	no	no pathology
060:2	m	30	mild	0	no	no	no pathology
061:2	f	37	mild	2	no	no	no pathology
062:2	m	84	mild	5	no	no	no pathology
064:2	m	67	mild	0	no	no	no pathology
144:2	f	71	mild	2	no	no	no pathology
173:2	f	56	mild	0	no	no	no pathology
202:2	m		mild	1	no	no	no pathology
231:3	m	83	mild	5	no	no	no pathology
238:3	m	64	mild	0	no	no	no pathology
255:3	f	29	mild	1	no	no	no pathology
260:1	f	54	mild	4	no	no	no pathology
291:2	f	19	mild	3	no	no	no pathology
304:1	f	30	mild	3	no	no	no pathology
309:2	m	21	mild	5	no	no	no pathology
312:3	m	45	mild	2	no	no	no pathology

Etiology: Biliary:0, Alcohol:1, ERCP:2, Drugs:3, Other:4, Unknown:5