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TITLE: Acute Pancreatitis as a Model to Predict Transition of Systemic Inflammation to Organ Failgure in Trauma & Critical Illness

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7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

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PITTSBURGH, PA 15213-3320

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U.S. Army Medical Research and Materiel Command
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14. ABSTRACT

Trauma, extensive burns, bacterial infections, and acute pancreatitis (AP) are common conditions of tissue injury and immune system activation that can result in the systemic inflammatory response syndrome (SIRS). Surprisingly, about half of the patients with SIRS quickly recover, while the others develop a multiorgan dysfunction syndrome (MODS). SIRS and MODS do not occur immediately: SIRS evolves over a 4-12 hour period, while MODS evolves over 12-24 hours. Vascular leak syndrome (VLS) is a critical component of the transition from SIRS to MODS. Understanding the mechanism by which SIRS triggers VLS and progresses to MODS is critical to correctly model disease course thereby aiding in treatment of patients. In this report, we analyzed the serum samples for proteins and fatty acids that will help to understand a mechanism for cytotoxicity to endothelial cells. The results demonstrate elevated cytokine, Ang-1, Ang-2 and activin levels in serum samples from patients with severe AP. Also, initial mass spectrometry findings show potential biomarkers that will be explored.

15. SUBJECT TERMS

Pancreatitis, systemic, inflammation, vascular leak, multiple organ dysfunction, biomarkers, endothelium, viability

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			USAMRMC
Unclassified	Unclassified	Unclassified	Unclassified	19	19b. TELEPHONE NUMBER *(include area code)*

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1. **INTRODUCTION:** Narrative that briefly (one paragraph) describes the subject, purpose and scope of the research.

The problem being addressed is the unknown mechanism(s) in patients with acute pancreatitis, multiple trauma, severe burn, or sepsis responsible for the unpredictable progression of systemic inflammation to the vascular leak syndrome (VLS), which in turn leads to multi-organ dysfunction syndrome (MODS). Our experimental approach is designed to understand and predict progression from systemic inflammation to MODS. The primary observation is that serum or plasma from patients with severe acute pancreatitis (AP) or trauma with VLS is toxic to endothelial cells.

2. **KEYWORDS:** Provide a brief list of keywords (limit to 20 words).

Pancreatitis, systemic, inflammation, vascular leak, multiple organ dysfunction, biomarkers, endothelium, viability

3. **ACCOMPLISHMENTS:** The PI is reminded that the recipient organization is required to obtain prior written approval from the awarding agency grants official whenever there are significant changes in the project or its direction.

What were the major goals of the project?
List the major goals of the project as stated in the approved SOW. If the application listed milestones/target dates for important activities or phases of the project, identify these dates and show actual completion dates or the percentage of completion.

Aim 1. Define the clinical setting in which SIRS progresses, and fails to progress, to VLS and MODS using molecular and clinical measures. (months 4-36)

Aim 2. Determine the effect of serum from patients with SIRS ±VLS as well as Ang-2 and other target molecules (identified in Aim 3) on human organ-derived endothelial cells in terms of morphology, gene activation, and mode of cell death. (months 4-36)

Aim 3. Identify serum molecule(s) that best predict specific in vitro changes in endothelial cells (Aim 2) as well as which molecule(s) and endothelial cell changes best predict clinical progression to MODS (Aim 1). (months 6-36)

What was accomplished under these goals?
For this reporting period describe: 1) major activities; 2) specific objectives; 3) significant results or key outcomes, including major findings, developments, or conclusions (both positive and negative); and/or 4) other achievements. Include a discussion of stated goals not met. Description shall include pertinent data and graphs in sufficient detail to explain any significant results achieved. A succinct description of the methodology used shall be provided. As the project progresses to completion, the emphasis in reporting in this section should shift from reporting activities to reporting accomplishments.

The ***first specific objective*** under **Aim 1** is to consent and enroll subjects into the study. There were 15 subjects enrolled into the study year 3. There were a total of 26 subjects in the study at the end of year 3. The ***second objective*** is to collect demographics, physiologic data, and pertinent medical information related to disease. The information from case report forms were entered into a secure database using Research Electronic Database Capture (REDCap) that can readily be converted into tabular format (Table 1). This allows us to easily pull de-identified data compiled from case report forms and medical records for evaluation and statistical comparisons.

Table 1. Clinical Information (select)

Record ID	Age	Gender	Etiology	BMI	Pain score at admission	SIRS score	Organ(s) failure	Pancreatic necrosis
001	68	Male	Biliary	33.4	N/A	2	Biliary, Renal, Cardiovascular	Yes
002	52	Female	Idiopathic/gallstones	40.9	10	3	Negative	Yes
003	39	Male	Hypertriglyceridemia	37.0	10	1	Respiratory	Yes
004	68	Male	Idiopathic	36.1	9	2	Respiratory	Yes
005	24	Male	Hypertriglyceridemia	32.3	8	2	Negative	No
006	48	Male	Alcoholism	23.8	10	2	Negative	Yes
007	79	Female	Gallstones	33.3	10	4	Respiratory, Renal	No
008	25	Female	Gallstones	29.9	10	1	Negative	No
009	37	Male	Alcoholic	23.3	10	2	Negative	No
010	66	Male	Biliary	32.5	10	3	Respiratory	Yes
011	49	Female	Idiopathic	24.2	10	3	Resiratory, Renal	No
012	52	Female	Biliary	40.6	8	3	Negative	No
013			Idiopathic	24.5			Negative	
014	28	Female	Post-ERCP	24.6	9	2	Negative	No
015	41	Male	Hypertriglyceridemia	31.8	9	2	Negative	No
016	59	Male	Biliary	44.4	10	3	Negative	Yes
017	42	Male	Hypertriglyceridemia	31.3			Renal	
018	53	Male	Biliary	44.4	5		Negative	No
019	37	Female	Hypertriglyceridemia	27.3			Negative	
020	34	Female	Hypertriglyceridemia	30.1			Negative	
021			Alcoholism	17.7			Negative	
022			Biliary	25.5			Negative	

Pain score – 1 – 10 (10 is worst); SIRS score – 0 to 4 depending on number of criteria met

The ***third objective*** of Aim 1 is to analyze the serum samples for candidate toxic factors and determine if they correlate with severity. The serum samples collected at days 1, 2, and 3 or first 3 time points collected from day of admission following enrollment into our study were analyzed for angiopoietin-1 (Ang-1) and activin A. Activin A is a cytokine that is a member of the TGF-β family. After ligand binding to its type II receptors (AVCR2A or ACVR2B) activin type I receptors (ACVR1 or ACVR1B) are activated through dimerization and phosphorylation, which subsequently leads to the activation of canonical SMAD- dependent and non-canonical SMAD-independent pathways. In inflammation, activin has been reported to have both pro- and anti-inflammatory functions ex vivo, resulting in either up or down regulation of a number of key inflammatory cytokines, including IL-6, IL-1β, or IL-10 in various human and murine cell types. In vivo, activin's reported actions are primarily pro-inflammatory. It increases very early in the inflammatory response and plays a central role in such diverse inflammatory conditions as IBD, asthma and viral infections. The results are shown in Table 2. As shown in Table 2, all of the activin A levels were found to be elevated in the subjects in comparison to levels reported in normal control subjects in the literature (0.11 ± 0.41 ng/ml (range 0.036 to 0.283 ng/ml)). We will also analyze control subject samples to make a comparison. Angiopoietin-1 plays a role in the modulation of blood vessel plasticity and contributes to vascular maintenance. Ang-1 enhances survival and migration of endothelial cells and induces neovascularization under both normal and pathogenic proangiogenic conditions. It is expressed in endothelial support cells, megakaryocytes,

and platelets. Both Ang-1 and Ang-2 are ligands for the endothelial cell receptor tyrosine kinase (Tie-2). Normal angiopoietin-1 levels are reported as 1,000 pg/ml (range 600-6,000 pg/ml) in the literature. The angiopoietin-1 levels in the severe acute pancreatitis patients tend to be high in comparison to these reported normal levels. We will also analyzed angiopoietin-1 levels in normal controls. It is interesting that both Ang-1 and Ang-2 are elevated in the patient serum samples. Activin from human samples was measured utilizing the activin A Quantikine ELISA (R&D Systems) following the manufacturer's instructions. All samples were run in duplicates after a 1:4 dilution in PBS. Ang-1 and Ang-2 were measured utilizing kits from Meso Scale Detection.

Table 2. Pro-inflammatory Cytokines and Angiopoietin Levels in Patient Samples

DOD.id	Day of blood draw	age.n	gender	IL-1β (pg/ml)	IL-6 (pg/ml)	IL-8 (pg/ml)	TNF-α (pg/ml)	MCP-1 (pg/ml)	Ang-2 (pg/ml)	Ang-1 (pg/ml)	Activin (ng/ml)
DOD001	1	68	M	19.57	4,938	8,217	28.37	9,558	217,368	3,939	1.704
DOD001	2	68	M	6.08	4,777	1,913	14.32	3,668	260,879	2,784	1.416
DOD001	3	68	M	2.72	1,807	691.8	6.47	3,470	105,143	6,523	0.968
DOD002	1	52	F	0.29	24.75	28.48	2.38	410.5	6,839	6,742	0.526
DOD002	2	52	F	0.20	15.01	19.42	1.85	318.6	10,858	7,328	1.746
DOD002	3	52	F	0.25	5.53	38.72	2.13	259.4	13,346	16,555	2.494
DOD003	1	39	M	0.72	76.18	43.63	1.75	1,100	17,401	12,535	3.270
DOD003	2	39	M	0.92	103.9	27.98	1.38	1,025	27,233	11,557	2.502
DOD003	3	39	M	0.57	35.37	33.92	2.15	540.9	29,601	18,103	2.600
DOD004	1	68	M	2.24	148.2	62.56	2.32	702.4	11,112	26,159	2.898
DOD004	2	68	M	1.30	231.2	61.27	2.53	656.8	18,095	23,282	2.700
DOD004	4	68	M	0.94	104.2	35.02	2.27	510.1	14,944	13,373	1.138
DOD005	3	24	M	0.42	9.41	5.85	0.99	NaN	7,968	10,485	2.586
DOD005	4	24	M	0.36	7.01	6.39	1.96	229.8	6,547	20,581	3.758
DOD005	5	24	M	0.44	8.07	5.88	2.72	309.5	6,347	17,362	2.890
DOD006	1	48	M	0.38	16.27	10.85	1.92	420.4	3,550	22,451	2.528
DOD006	2	48	M	0.54	56.23	13.90	1.56	323.5	4,701	20,035	2.626
DOD006	3	48	M	0.50	180.5	37.52	2.08	456.6	5,859	21,622	2.468
DOD007	1	79	F	0.42	32.62	58.68	4.02	866.7	22,945	18,062	2.516
DOD007	2	79	F	2.60	320.8	187.6	14.34	1,103	61,764	11,563	2.030
DOD007	3	79	F	1.13	323.8	113.4	8.72	950.5	56,886	4,146	3.534
DOD008	2	25	F							9,467	2.690
DOD009	2	36	M	0.15	59.66	13.68	1.59	563.5	3,001	13,596	2.596
DOD009	3	36	M	0.16	37.09	8.06	1.92	341.7	2,912	13,005	2.562
DOD009	4	36	M							7,429	1.752
DOD010	1	65	M							16,735	1.600
DOD010	2	65	M							6,016	1.314
DOD010	3	65	M							11,956	2.614
DOD011	1	49	F							2,722	2.108
DOD011	2	49	F							236.1	1.812
DOD011	3	49	F							1,722	1.512
DOD012	1	51	F							12,580	1.272
DOD012	2	51	F							13,322	1.008
DOD012	3	51	F							14,244	0.740
DOD013	1	37	F							43,057	1.238
DOD013	2	37	F							28,805	3.114
DOD013	3	37	F							18,361	2.324
DOD014	1	28	F							9,041	1.414
DOD014	2	28	F							10,079	1.634
DOD014	3	28	F							11,105	1.708
DOD015		41	M								
DOD016		59	M								

Additionally, several of the serum samples collected that showed significant toxicity to the endothelial cells were analyzed for serum free fatty acids (FFA) in order to evaluate the different levels and potentially correlate them to the level of toxicity, and possibly other factors that are being monitored from the clinical data. Both saturated fatty acids (SFA) i.e. palmitic (16:0) and stearic (18:0) and unsaturated fatty acids (UFA); monounsaturated, i.e. palmitoleic (16:1), oleic (18:1), and polyunsaturated, i.e. linoleic (18:2) were measured. The FFA were analyzed using an Agilent Technologies 6890N Network gas chromatography (GC) System with Flame Ionizaton Detector. The serum samples were extracted with isopropanol-heptane-hydrochloric acid. Heptane and water were added and the tubes vortexed. Tubes were then centrifuged and the upper phase (heptane) transferred into clean screw top tubes and dried in a SpeedVac centrifugal concentrator. FFA were then derivatized with dimethylamine and diisopropylethylamine using the Deoxo-Fluor reagent (Sigma Aldrich). The results indicate severe subject DOD001 had very high FFA with average palmitic acid of 431.2 μM, palmitoleic acid of 31.1 μM, stearic acid of 133.4 μM, oleic acid of 344 μM, and linoleic acid of 258.6 μM. The other 2 subjects with severe pancreatitis analyzed (DOD002, DOD004, DOD005) had averages of 253, 22.7, 83.5, 166, 61.5 μM palmitic acid, palmitoleic acid, stearic acid, oleic acid, and linoleic acid respectively. Subject DOD003 with moderate pancreatitis had 295, 19.4, 105, 180, 74.7 μM palmitic acid, palmitoleic acid, stearic acid, oleic acid, and linoleic acid respectively. A subpopulation of subjects with mild pancreatitis had an average of 174, 13.6, 58.2, 150, 97.8 μM palmitic acid, palmitoleic acid, stearic acid, oleic acid, and linoleic acid respectively. Control subjects without pancreatitis had 106, 7.7, 40.8, 62.0, 45.3 μM palmitic acid, palmitoleic acid, stearic acid, oleic acid, and linoleic acid respectively. There is a decrease in FFA between the severe/moderate and mild control subjects.

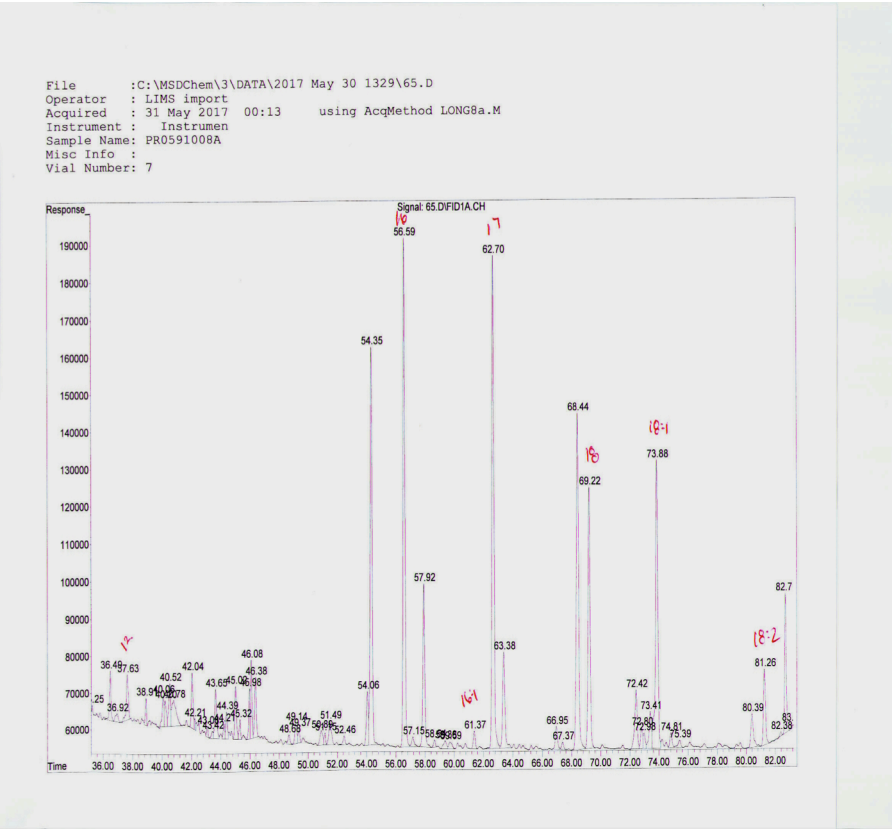
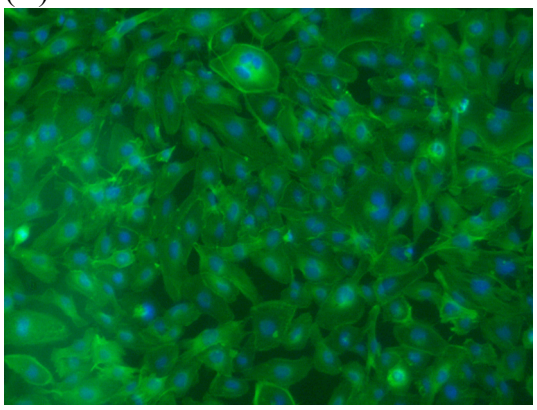


Figure 1. Gas Chromatogram of Free Fatty Acids. The Free Fatty Acids are separated on a Supelco SP 2380 capillary column. The retention times of the FFA are palmitic acid 56.59 min, palmitoleic acid 61.37 min, heptadecanoic acid (Internal Standard) 62.70 min, stearic acid 69.22 min, oleic acid 73.88 min, and linoleic acid 81.26 min.

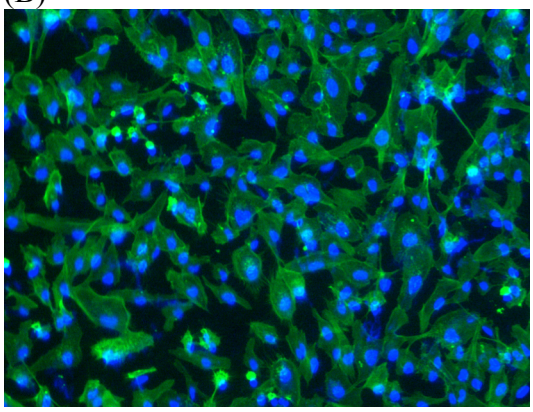
The specific objective under **Aim 2** includes assessing the effect of serum from subjects enrolled in the study on primary endothelial cells. The morphology and physiology of newly isolated cells following treatment with the serum samples from subjects were assessed with F-actin stress fiber staining with phalloidin. The figures below show that the morphology of the isolated vascular endothelial cells change following 24 hour treatments with serum collected on days 1, 2, and 3 from subject DD011 with severe acute pancreatitis. The figures show how the serum from the severe acute pancreatitis subject causes the F-actin stress fibers to thicken leading to the abnormal appearance of the cells.

Figure 2. F-actin fibers stained with phalloidin (green) and nuclei stained with 4',6-Diamidino-2-phenylindole dihydrochloride (DAPI) in human intestinal vascular endothelial cells in normal growth medium containing 20% normal control serum (A) and treated 20% DOD011 Day 1 serum (B), 20% DOD011 Day 2 (C), 20% DOD011 Day 3 (D). Images demonstrate thickening of stress fibers due to activation of endothelial cells. 10X magnification.

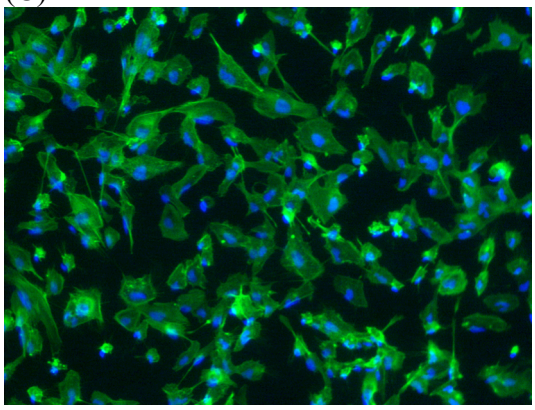
(A)



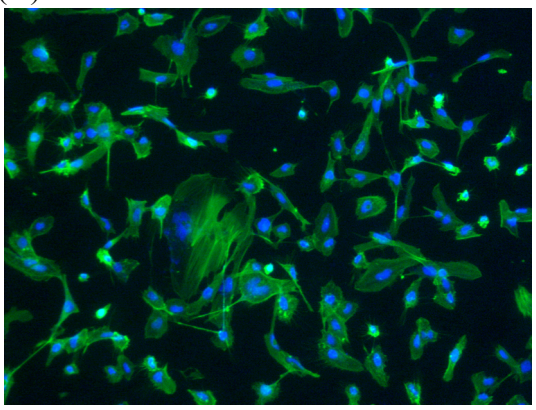
(B)



(C)



(D)



The endothelial cell viability (MTT assay) and cytotoxicity (LDH release assay) caused by the patient serum samples was evaluated. As can be seen in Figures 3 and 4 not all sera samples decrease cell viability or induce cytotoxicity.

Figure 3. **MTT Assay**. The chart shows that cell viability decreases when primary human intestinal endothelial cells are treated with 20% serum from some of the severe acute pancreatitis patients (DOD001, DOD002, DOD004) in comparison with cells treated with 20% normal control serum.

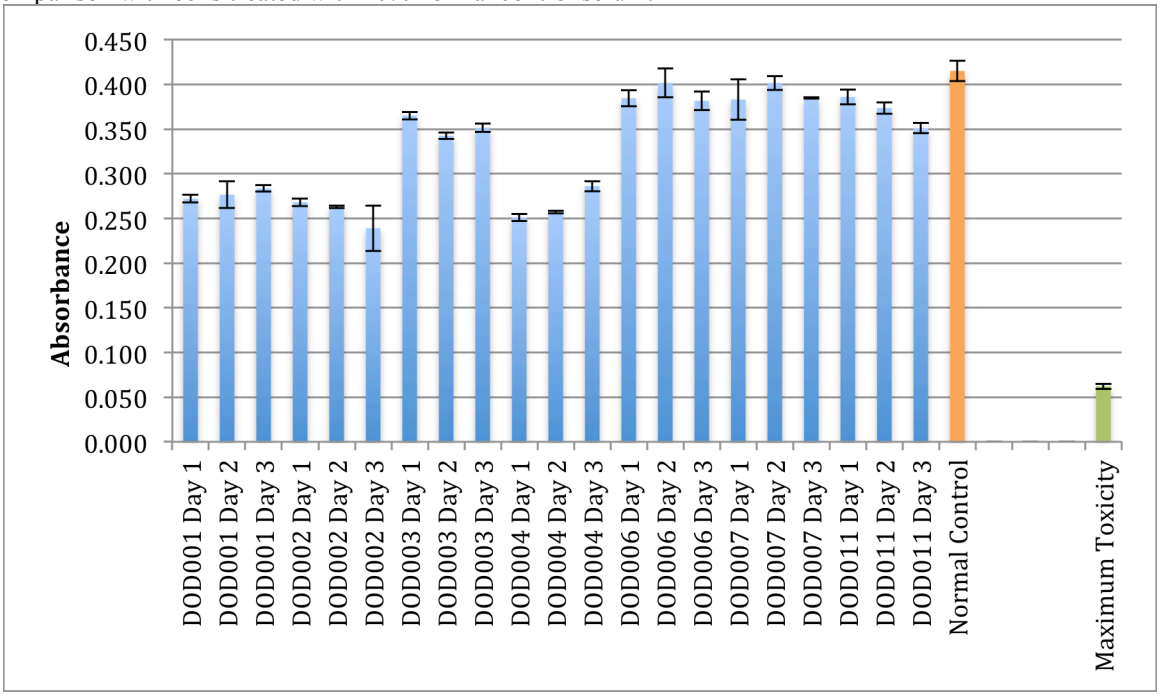
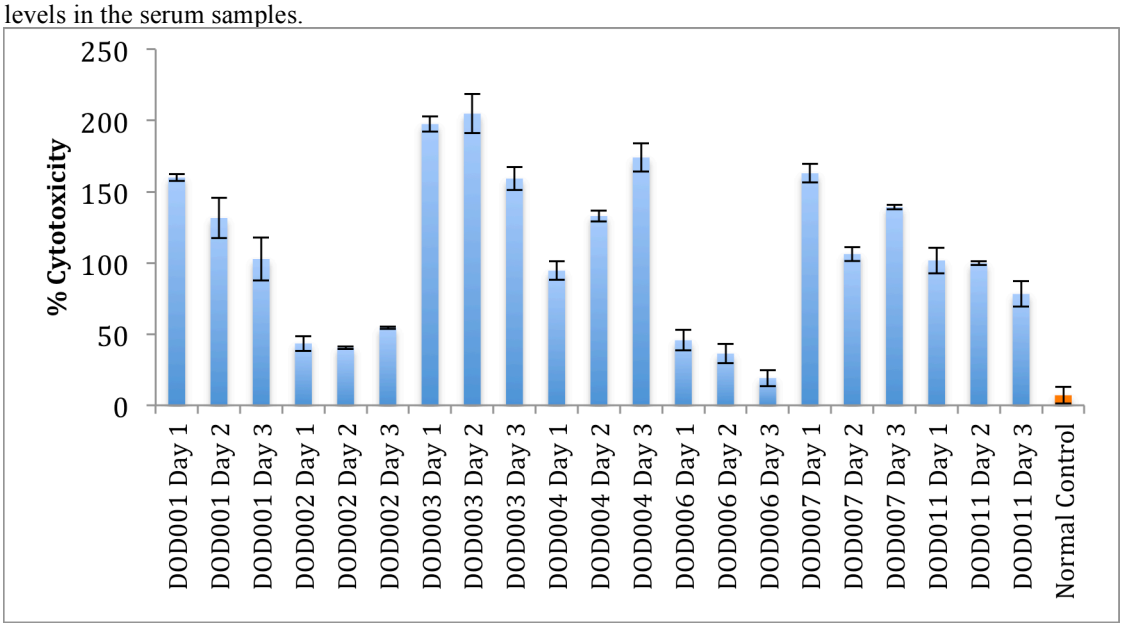


Figure 4. **LDH Release Assay**. The chart shows that cytotoxicity increases when primary human intestinal endothelial cells are treated with 20% serum from severe acute pancreatitis patients in comparison with cells treated with 20% normal control serum. The lactate dehydrogenase release assay measures the release of LDH from the endothelial cells into the medium. This indicates a loss of integrity of the cellular membrane. We will need to also measure the LDH levels in the serum samples.



What opportunities for training and professional development has the project provided?
If the project was not intended to provide training and professional development opportunities or there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe opportunities for training and professional development provided to anyone who worked on the project or anyone who was involved in the activities supported by the project. "Training" activities are those in which individuals with advanced professional skills and experience assist others in attaining greater proficiency. Training activities may include, for example, courses or one-on-one work with a mentor. "Professional development" activities result in increased knowledge or skill in one's area of expertise and may include workshops, conferences, seminars, study groups, and individual study. Include participation in conferences, workshops, and seminars not listed under major activities.

Nothing to report.

How were the results disseminated to communities of interest?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how the results were disseminated to communities of interest. Include any outreach activities that were undertaken to reach members of communities who are not usually aware of these project activities, for the purpose of enhancing public understanding and increasing interest in learning and careers in science, technology, and the humanities.

Nothing to report.

What do you plan to do during the next reporting period to accomplish the goals?

If this is the final report, state "Nothing to Report."

Describe briefly what you plan to do during the next reporting period to accomplish the goals and objectives.

Patient recruitment/enrollment into the project will be a priority. Serum will be tested for effect on endothelial cell viability. In upcoming experiments, methods to define mode of cell death (Aim 2) will be performed. All of the patient samples will be analyzed by mass spectrometry to determine potential biomarkers (Aim 3). Finally, recombinant Ang-2 effects on the endothelial cells will be studied and known blockers of Ang-2 will be studied to reverse the toxicity to the endothelial cells.

4. **IMPACT:** Describe distinctive contributions, major accomplishments, innovations, successes, or any change in practice or behavior that has come about as a result of the project relative to:

What was the impact on the development of the principal discipline(s) of the project?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how findings, results, techniques that were developed or extended, or other products from the project made an impact or are likely to make an impact on the base of knowledge, theory, and research in the principal disciplinary field(s) of the project. Summarize using language that an intelligent lay audience can understand (Scientific American style).

Nothing to report.

What was the impact on other disciplines?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how the findings, results, or techniques that were developed or improved, or other products from the project made an impact or are likely to make an impact on other disciplines.

Nothing to report

What was the impact on technology transfer?

If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe ways in which the project made an impact, or is likely to make an impact, on commercial technology or public use, including:

- *transfer of results to entities in government or industry;*
- *instances where the research has led to the initiation of a start-up company; or*
- *adoption of new practices.*

Nothing to report.

What was the impact on society beyond science and technology?

If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how results from the project made an impact, or are likely to make an impact, beyond the bounds of science, engineering, and the academic world on areas such as:

- *improving public knowledge, attitudes, skills, and abilities;*
- *changing behavior, practices, decision making, policies (including regulatory policies), or social actions; or*
- *improving social, economic, civic, or environmental conditions.*

Nothing to report.

5. **CHANGES/PROBLEMS:** The PD/PI is reminded that the recipient organization is required to obtain prior written approval from the awarding agency grants official whenever there are significant changes in the project or its direction. If not previously reported in writing, provide the following additional information or state, "Nothing to Report," if applicable:

Nothing to report.

Actual or anticipated problems or delays and actions or plans to resolve them
Describe problems or delays encountered during the reporting period and actions or plans to resolve them.

The recruitment of patients into the study picked up this year. We are confident that we will be able to complete enrollment this year and complete the project.

Changes that had a significant impact on expenditures
Describe changes during the reporting period that may have had a significant impact on expenditures, for example, delays in hiring staff or favorable developments that enable meeting objectives at less cost than anticipated.

We replaced our Research Nurse Coordinator (Kelley Woods) in November 2016 which helped with enrollment.

Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents
Describe significant deviations, unexpected outcomes, or changes in approved protocols for the use or care of human subjects, vertebrate animals, biohazards, and/or select agents during the reporting period. If required, were these changes approved by the applicable institution committee (or equivalent) and reported to the agency? Also specify the applicable Institutional Review Board/Institutional Animal Care and Use Committee approval dates.

Significant changes in use or care of human subjects

Nothing to report.

Significant changes in use or care of vertebrate animals

Not applicable.

Significant changes in use of biohazards and/or select agents

Not applicable.

6. **PRODUCTS:** List any products resulting from the project during the reporting period. If there is nothing to report under a particular item, state "Nothing to Report."

- **Publications, conference papers, and presentations**
  Report only the major publication(s) resulting from the work under this award.

  Journal publications. *List peer-reviewed articles or papers appearing in scientific, technical, or professional journals. Identify for each publication: Author(s); title; journal; volume: year; page numbers; status of publication (published; accepted, awaiting publication; submitted, under review; other); acknowledgement of federal support (yes/no).*

  Nothing to report.

  Books or other non-periodical, one-time publications. *Report any book, monograph, dissertation, abstract, or the like published as or in a separate publication, rather than a periodical or series. Include any significant publication in the proceedings of a one-time conference or in the report of a one-time study, commission, or the like. Identify for each one-time publication: author(s); title; editor; title of collection, if applicable; bibliographic information; year; type of publication (e.g., book, thesis or dissertation);*

status of publication (published; accepted, awaiting publication; submitted, under review; other); acknowledgement of federal support (yes/no).

Nothing to report.

Other publications, conference papers and presentations. *Identify any other publications, conference papers and/or presentations not reported above. Specify the status of the publication as noted above. List presentations made during the last year (international, national, local societies, military meetings, etc.). Use an asterisk (*) if presentation produced a manuscript.*

Abstracts for presentations at meetings:
American College of Gastroenterology Tuesday, October 15, 2017: Severe Acute Pancreatitis and Shock: Are High Angiopoetin-2 Levels causing endothelial cell dysfunction and a Vascular Leak Syndrome? Annette Wilson PhD, Anna Evans Phillips MD, Kelley Woods, Kimberly Stello, David Binion and David C. Whitcomb; Poster

- **Website(s) or other Internet site(s)**
  List the URL for any Internet site(s) that disseminates the results of the research activities. A short description of each site should be provided. It is not necessary to include the publications already specified above in this section.

Nothing to report.

- **Technologies or techniques**

Identify technologies or techniques that resulted from the research activities. Describe the technologies or techniques were shared.

Nothing to report.

- **Inventions, patent applications, and/or licenses**

  Identify inventions, patent applications with date, and/or licenses that have resulted from the research. Submission of this information as part of an interim research performance progress report is not a substitute for any other invention reporting required under the terms and conditions of an award.

Nothing to report.

- **Other Products**

  Identify any other reportable outcomes that were developed under this project. Reportable outcomes are defined as a research result that is or relates to a product, scientific advance, or research tool that makes a meaningful contribution toward the understanding, prevention, diagnosis, prognosis, treatment and /or rehabilitation of a disease, injury or condition, or to improve the quality of life. Examples include:

  - *data or databases;*
  - *physical collections;*
  - *audio or video products;*
  - *software;*
  - *models;*
  - *educational aids or curricula;*
  - *instruments or equipment;*
  - *research material (e.g., Germplasm; cell lines, DNA probes, animal models);*
  - *clinical interventions;*
  - *new business creation; and*
  - *other.*

Nothing to report.

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?

Name: David C. Whitcomb, MD
Project Role: PI
Nearest person month(s) worked: 2.4 months
Contribution to Project: Dr. Whitcomb oversaw all research in this project. Weekly research meetings were held to disseminate progress. In addition, interviewed candidates for the Nurse Research Coordinator position.

Name: David G. Binion, MD
Project Role: Co-Investigator
Nearest person month(s) worked: 1.2 months
Contribution to Project: Dr. Binion provided assistance with experiments in this project and participates in research meetings.

Name: Annette S. Wilson, PhD
Project Role: Laboratory Manager
Nearest person month(s) worked: 8.4 months
Contribution to Project: Dr. Wilson coordinated the experiments and performed imaging, chromatography, ELISA assays, and data analysis. She participates in the weekly research meetings. In addition, Dr. Wilson assisted Dr. Whitcomb with writing the IRB renewal application.

Name: Kelley Woods, RN
Project Role: Research Nurse Coordinator
Nearest person month(s) worked: 3 months
Contribution to Project: Mrs. Woods has consented all patients currently in the study. She has transported the blood samples to the research lab and assisted in processing, aliquotting, and storing samples. She attends the weekly research meetings.

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?

Nothing to Report.

What other organizations were involved as partners?

Nothing to Report.

8. SPECIAL REPORTING REQUIREMENTS

COLLABORATIVE AWARDS: For collaborative awards, independent reports are required from BOTH the Initiating Principal Investigator (PI) and the Collaborating/Partnering PI. A duplicative report is acceptable; however, tasks shall be clearly marked with the responsible PI and research site. A report shall be submitted to **https://ers.amedd.army.mil** for each unique award.

QUAD CHARTS: If applicable, the Quad Chart (available on https://www.usamraa.army.mil) should be updated and submitted with attachments.

9. APPENDICES:

Attach all appendices that contain information that supplements, clarifies or supports the text. Examples include original copies of journal articles, reprints of manuscripts and abstracts, a curriculum vitae, patent applications, study questionnaires, and surveys, etc.

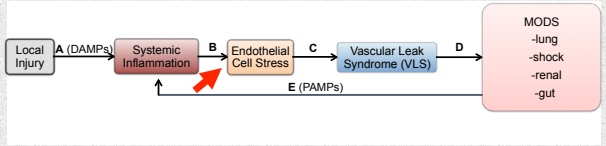
Severe acute pancreatitis and shock: Are high angiopoetin-2 levels causing endothelial cell dysfunction and vascular leak syndrome?

Annette Wilson, Anna Evans Phillips, Kelley Woods, Kimberly Stello, David G. Binion, David C. Whitcomb

Division of Gastroenterology, Hepatology and Nutrition, University of Pittsburgh School of Medicine, Pittsburgh, Pennsylvania, USA

Introduction

Acute pancreatitis (AP) is an acute inflammatory syndrome originating in the pancreas with variable progression from injury, to local inflammation, to systemic inflammation to vascular leak syndrome and multi-organ dysfunction syndrome (MODS). Systemic inflammation, measured as the systemic inflammatory response syndrome (SIRS), appears to be necessary, but not sufficient to cause MODS. We previously demonstrated that angiopoetin-2 (Ang-2), an endothelial cell paracrine hormone associated with local vascular leak following injury, was significantly higher on admission in patients who developed persistent organ failure compared with those who did not (PMID: 20461065). This suggested that endothelial cell dysfunction linked SIRS with MODS via VLS with pulmonary edema and hypovolumea/hemoconcentration as primary clinical signs. However, it was not clear whether Ang-2 was a consequence, or mediator of endothelial cell injury.



Severity categories following the revision of Atlanta Classification

Acute pancreatitis severity	*Organ failure and local or systemic complications*
Mild acute pancreatitis	- No organ failure - No local or systemic complications
Moderately severe acute pancreatitis	- Transient organ failure (resolves in 48 hours) - Local or systemic complications without persistent organ failure
Severe acute pancreatitis	- Persistent organ failure (single or multiple)

Methods

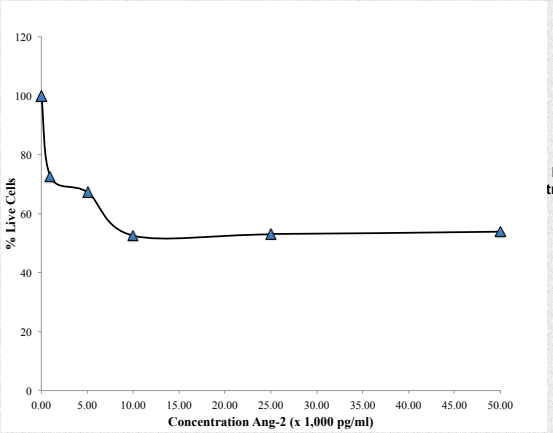
In a follow-up clinical study, clinical information and blood was collected from patients with severe AP at admission and for 7 days. Serum was assayed for Ang-2 levels (MSD). Human vascular endothelial cells were cultured and treated with serum from severe AP patients and synthetic Ang-2 (R&D Systems) at concentrations of 0 (control) to 50,000 pg/ml. Cell morphology and viability were measured by lactate dehydrogenase release assay, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide (MTT) assay, phalloidin fluorescent staining. Caspase 3/7 activation was also measured after 4 hour treatments.

Results

Ang-2 levels in patients with severe AP ranged from 3,000 to 260,000 pg/ml, with normal values in our controls being <2,000 pg/ml. The addition of serum from patients with severe, but not mild AP induced in endothelial cell stress and death after 24 hour treatments. Synthetic Ang-2 began decreasing cell viability at concentrations of 5,000 ng/ml with IC 50 of 42,500 ng/ml as measured by the MTT assay. Caspase 3/7 was not activated by Ang-2. Ang-2 alone did not appear to cause an endothelial stress response, or apoptosis.

Inclusion and exclusion criteria

Inclusion criteria	*Exclusion criteria*
- Patients 18 years of age or older - Diagnosis of acute pancreatitis (a) History of sudden onset abdominal pain (b) Elevation of amylase or lipase > 3 times normal upper clinical limits (c) Characteristic signs on abdominal imaging - Evidence of SIRS defined by 2 or more of the following features: (a) Heart rate over 90 bpm (b) Body Temp < 36 or > 38°C (c) Tachypnea > 20 breaths per minute or $PaCO_2$ < 32 mm Hg (d) WBC < 4,000 or >12,000 cell/mm^3 or > 10% immature neutrophils (bands)	- Time elapse between initial AP symptoms and collection of first serum sample > 48 hrs - Patients with chronic pancreatitis and pancreatic insufficiency based on clinical history - Pre-existing chronic renal insufficiency requiring dialysis - Pre-existing liver disease - Pre-existing immune deficiency - Pre-existing pulmonary disease - Pre-existing cardiovascular disease - Pancreatic cancer and any other forms of cancer with life expectancy < 6 months



MTT Assay – Endothelial cells treated with Ang-2

Conclusions

- This is the first study to demonstrate that Ang-2, in levels seen during severe AP in humans, result in endothelial cell death.
- These results provide insight into possible mechanism of severe AP with VLS and prolonged recovery.
- Ang 2 levels are extremely elevated in patients with severe acute pancreatitis.
- The mechanism of cell death, and the potential contributions of other factors in this process are important for future development of effective interventions to prevent or limit MODS.

Clinical data on acute pancreatitis patients

Patient no.	Age (y)	Gender	Etiology	BMI	Organ(s) Failure	Pain score	Pancreatic necrosis	SIRS score	Day No.	Ang-2 (pg/mL)
1	68	M	Biliary	33.4	Biliary, Renal, Cardiovascular		Yes	2	1	217,369
									6	82,558
2	52	F	Idiopathic	40.9	Negative	10	Yes	3	1	6,840
									4	18,517
3	39	M	Post-ERCP	37.0	Respiratory	10	Yes	1	1	17,402
									6	25,363
4	68	M	Idiopathic	36.1	Respiratory	9	Yes	2	1	11,112
									7	20,208
5	24	M	HTG	32.3	Negative	8	No	2	3	7,968
									7	2,955
6	48	M	Alcoholism	23.8	Negative	10	Yes	2	1	3,551
									7	7,244
7	79	F	Biliary	33.3	Respiratory, Renal	10	No	4	1	22,945
									6	25,026
9	37	M	Alcoholism	23.5	Negative	10	No	2	2	3,002
									3	2,913
11	49	F	Idiopathic	24.2	Respiratory, Renal	10	No	3	1	61,292
									7	25,067
12	52	F	Biliary	40.6	Negative	10	No	3	1	14,640
									7	28,011
14	28	F	Post-ERCP	24.6	Negative	9	No	2	1	42,652
									6	27,694
15	41	M	HTG	31.8	Negative	9	No	2	1	28,214
									3	27,369
16	59	M	Biliary	25.2	Negative	10	Yes	3	1	7,856
									4	13,411
18	53	M	Biliary	44.4	Negative	5	No		1	34,272
									4	30,920

HTG: Hypertriglyceridemia; HR: Heart rate; Hct: Hematocrit; Pain score – 1 to 10 (10 is worst); SIRS score – 0 to 4 depending on number of criteria met; Ranson score – 0 to 5 depending on number of criteria met; Apache II scores – 0 to 16 depending on criteria met; Norm values: Ang 2 = 1,075 ± 228.2 pg/mL

Acknowledgements

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