Antimicrobial Defence in Human Body Fluids - High Antimicrobial Peptide Expression in Postoperative Pleural Fluid and Stressed Mononuclear Cells

Doctoral Viva

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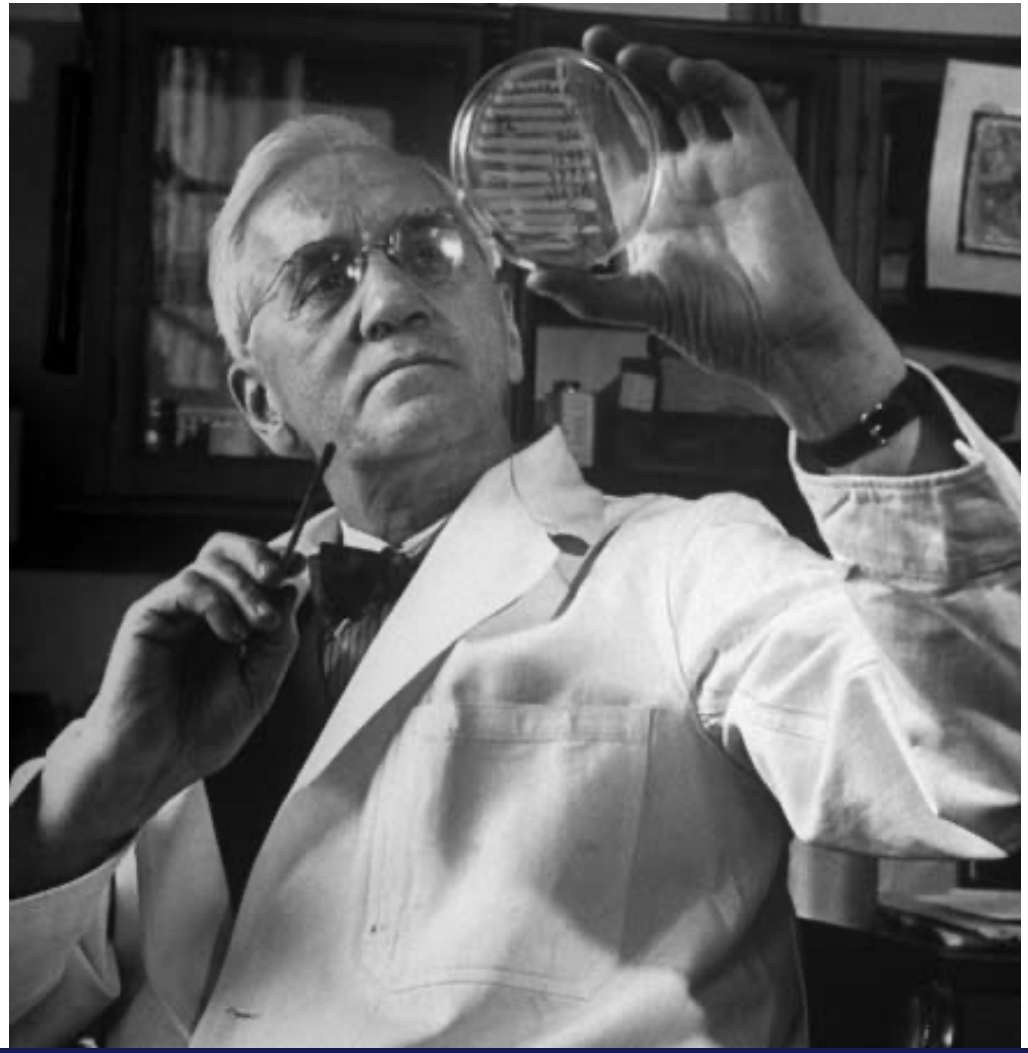
Vienna, Juli 2020

Sir. Alexander Fleming 1881-1955

- Lysozyme in human tears in 1922
- Accidental contamination of a staphylococci culture plate by a mould named “Penicillium” in 1928
- The Nobel Prize in Physiology or Medicine, December 10, 1945



https://www.nobelprize.org/prizes/medicine/1945/fleming/biographical/

Nobel Lecture, December 11, 1945

I had since the war of 1914-1918 been interested in antiseptics and in 1924 I described what I think is probably the best experiment I ever did. This showed up in a dramatic fashion the relative activity of a chemical on bacteria and on human leucocytes.

Normal human blood has a strong bactericidal power on the ordinary cocci, e.g. staphylococci and streptococci, but this power is completely lost if the leucocytes are removed from the blood. If defibrinated blood is infected with a small number of staphylococci (say 4,000 per cc.) and incubated in a capillary space - a slide cell or a capillary tube - the cocci which survive grow out into colonies which can easily be enumerated. But only about 5 per cent grow out. If however, phenol is added to B concentration of 1 in 600 all the cocci grow out freely. Here the phenol in a concentration which does not interfere with bacterial growth has destroyed the leucocytes which constitute one of our most powerful defences against infection (see

https://www.nobelprize.org/uploads/2018/06/fleming-lecture.pdf

Antimicrobial Peptides (AMPs)

- Component of the hosts defence mechanism
- Prokaryotes (bacteria) and eukaryotes (plants, insects, and animals)
- Over 2500 oligopeptides (10 - >50 amino acids)
- Broad spect. AMA against: gram-neg., gram-pos. bacteria, yeasts, fungi, viruses, and even cancer cells
- Direct antimicrobial, chemotactic, immunomodulatory, angiogenetic activity…

J Exp Med. 1939 Jun 30; 70(1): 1–10

Antimicrobial Defence of Human Body Fluids

Major cardio-thoracic surgery → Thoracotomy

- Connection between Lung and Pleura
  - Lung: ~ 50% pathogen colonisation
- Post-op empyema < 5%

European Journal of Cardio-Thoracic Surgery, 2011, 40(2)309-319

Chest, 2005, 128(3):1571-9

Antimicrobial Defence of Human Body Fluids

› Ann Thorac Surg. 2014 Sep;98(3):1042-50. doi: 10.1016/j.athoracsur.2014.04.106. Epub 2014 Jul 16.

Antimicrobial Peptides Are Highly Abundant and Active in Postoperative Pleural Drainage Fluids

Konrad Hoetzenecker [1], Moritz Hochdaninger [1], Denise Traxler [2], Maria Gschwandtner [3], Mohammad Mahdi Kasiri [2], Andreas Mitterbauer [2], Thomas Schweiger [2], Balazs Hegedus [1], Walter Klepetko [1], Erwin Tschachler [3], Hendrik J Ankersmit [4], Michael Mildner [3]

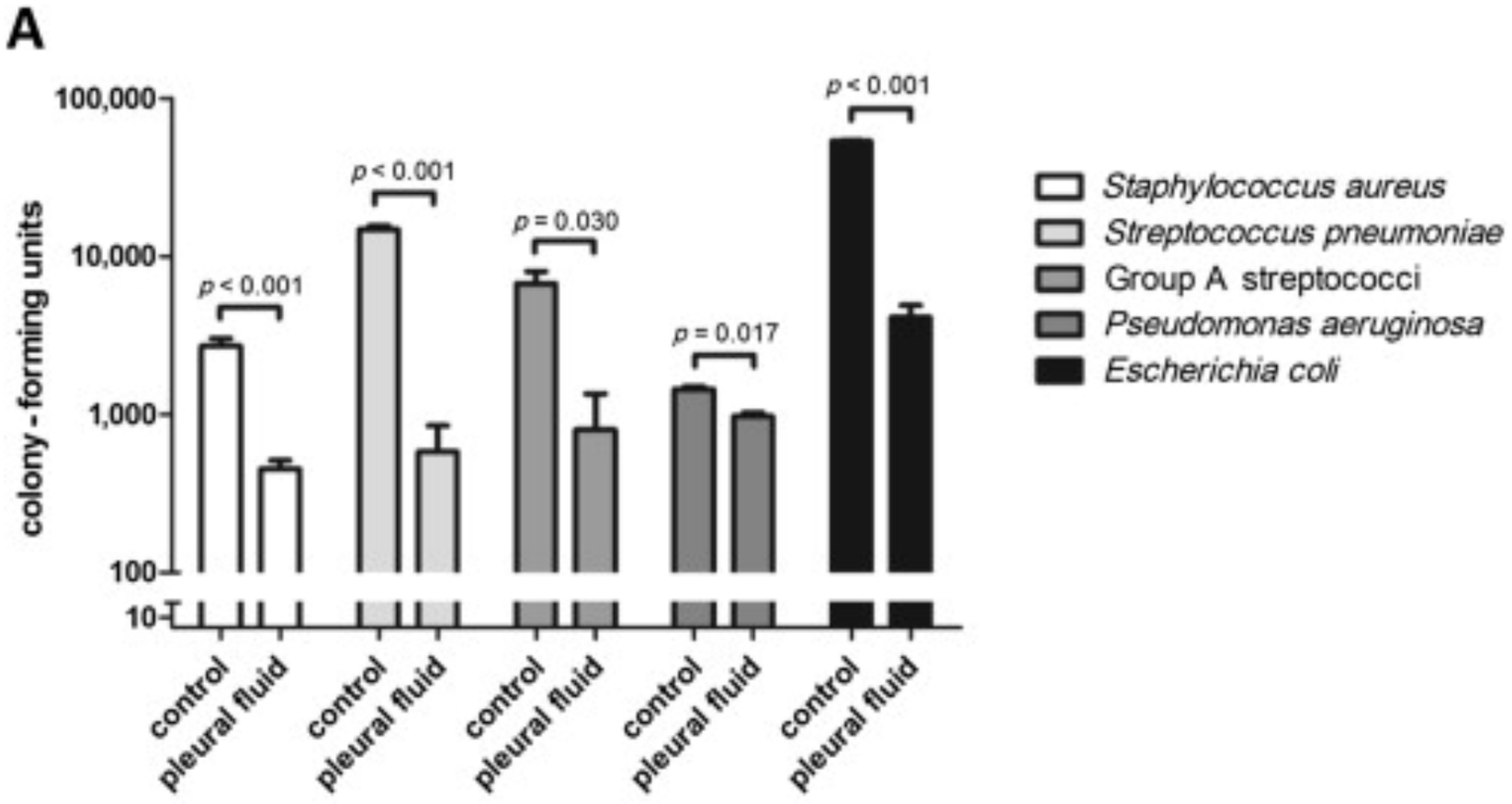
Methods

- Collection of:
  - Pleural fluid
  - parietal pleura samples
- Applied techniques:
  - Microdilution assay for antimicrobial activity
  - ELISA: AMPs
  - PCR: pleural fluid, parietal pleura
  - Immunohistochemical staining on parietal pleura
  - Flow Cytometry: cellular components of Pleura fluid

Ann Thorac Surg 2014, 98(3)1042-1050

Results

Antimicrobial activity of post-Op pleural fluids



Ann Thorac Surg 2014, 98(3)1042-1050

Results

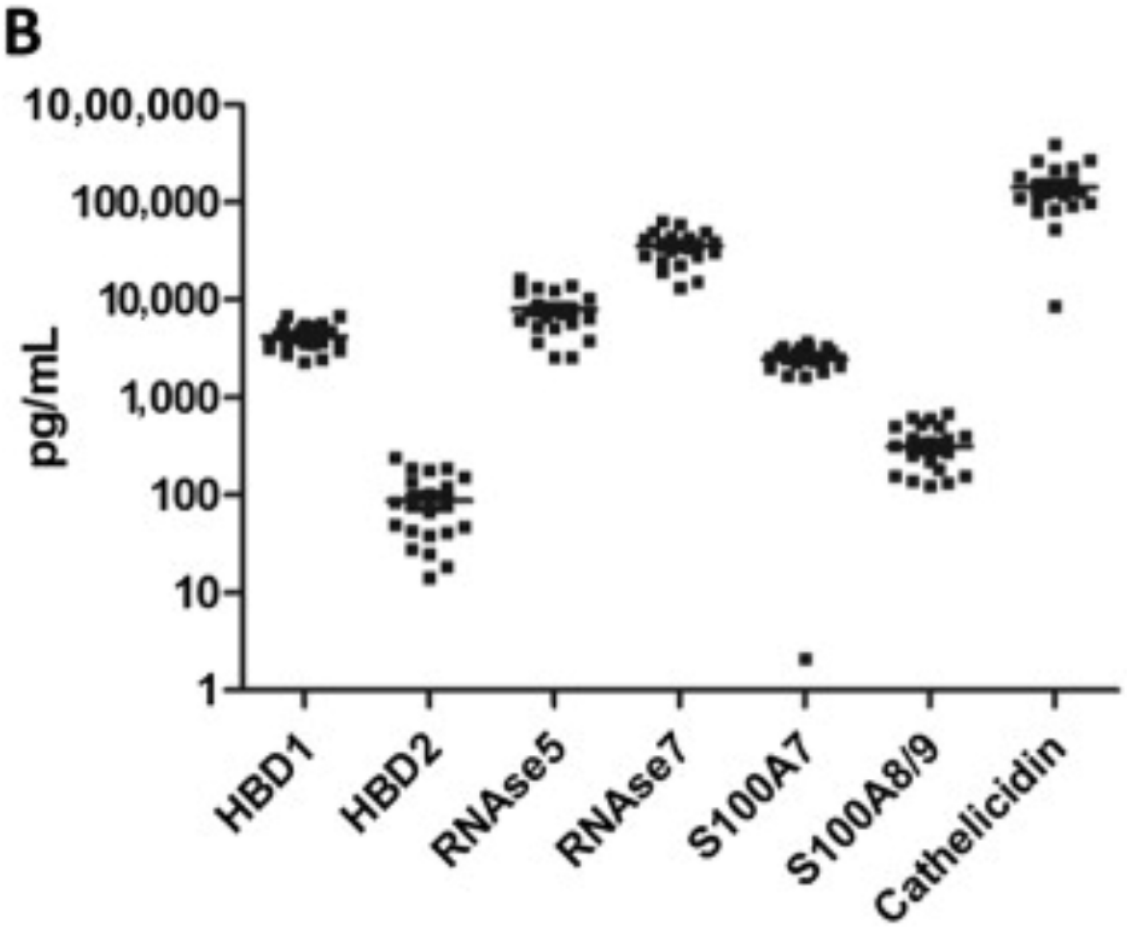
AMP concentration in post-Op pleural fluid

Organism	Antibiotic Coverage	No Antibiotic Coverage	*p* Value
Staphylococcus aureus	86.6±1.3%	53.5±3.8%	<0.001
Streptococcus pneumonmiae	97.4±1.3%	67.0±3.8%	<0.001
Group A streptococci	95.5±4.3%	0.0±0.0%	<0.001
Pseudomonas aeruginosa	30.5±4.0%	23.4±7.2%	0.628
Escherichia coli	86.8±3.7%	62.4±2.2%	0.081

Ann Thorac Surg 2014, 98(3)1042-1050

Results

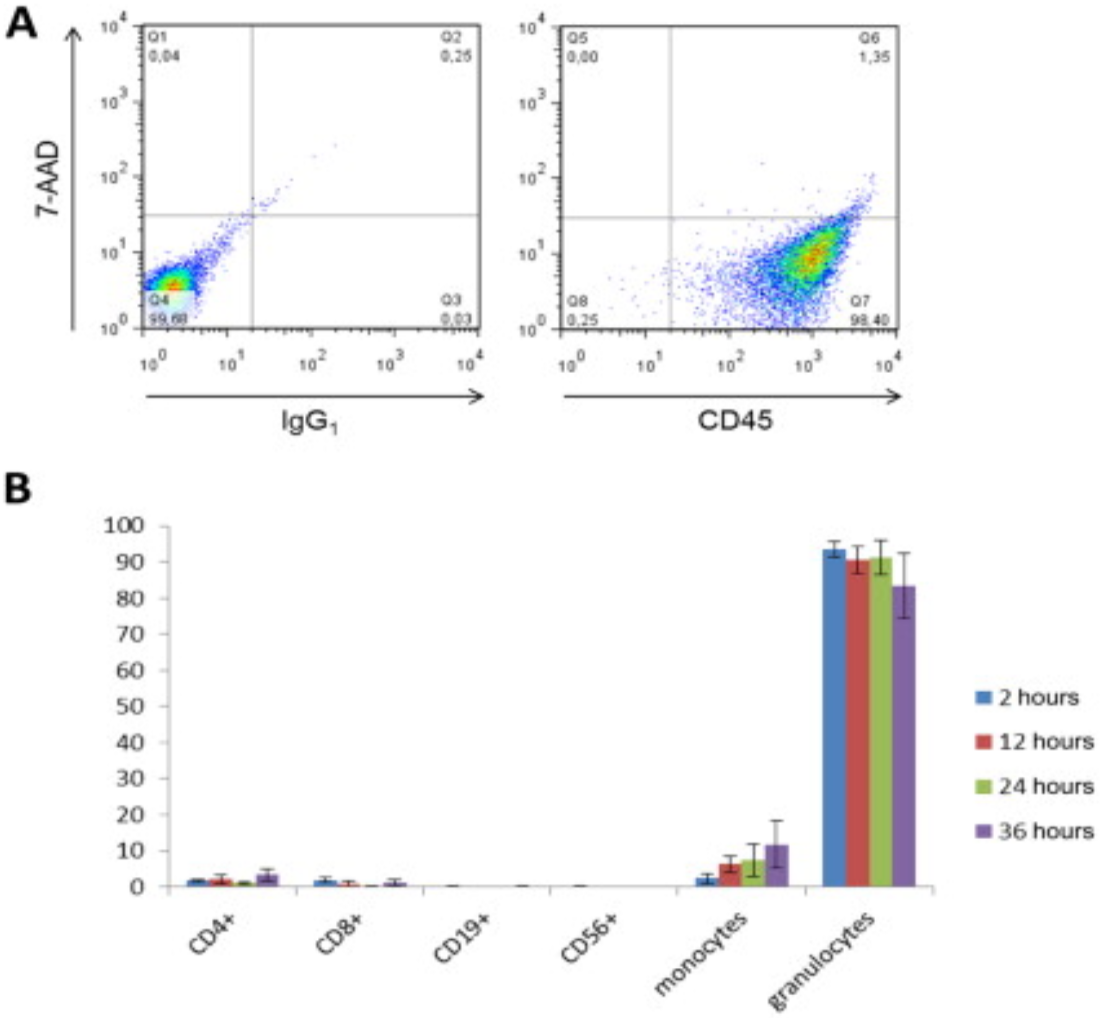
AMP concentration in post-Op pleural fluid



Ann Thorac Surg 2014, 98(3)1042-1050

Results

Search for the AMP source: Flow Cytometry



Ann Thorac Surg 2014, 98(3)1042-1050

Summery

For the first time:

post-Op pleural fluid

1. Potent antimicrobial activity
2. Gram-positive and gram-negative bacteria
3. AMPs

Ann Thorac Surg 2014, 98(3)1042-1050

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Blood. 2000 Nov 1;96(9):3086-93.

The Human Antimicrobial and Chemotactic Peptides LL-37 and Alpha-Defensins Are Expressed by Specific Lymphocyte and Monocyte Populations

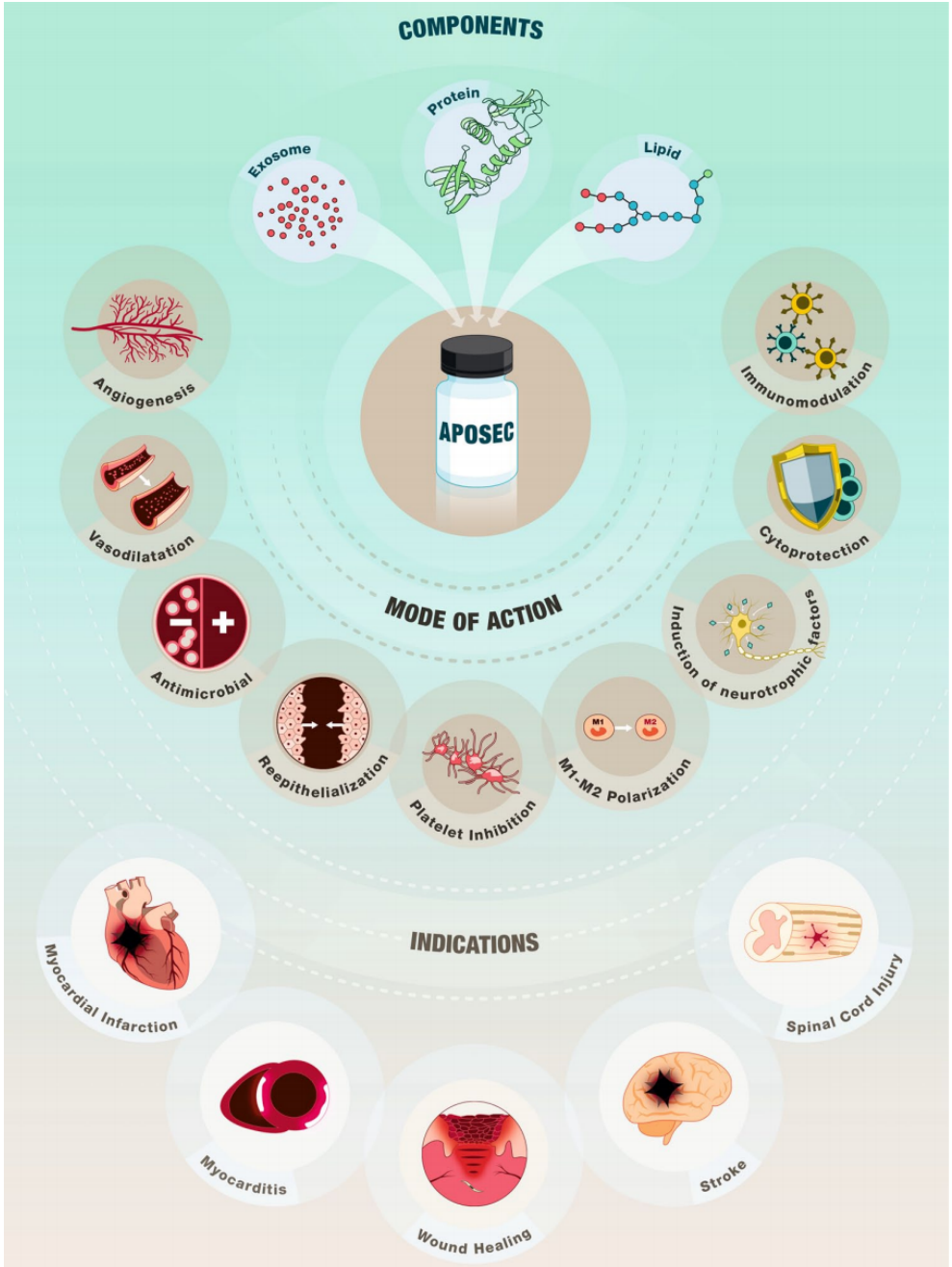
B Agerberth [1], J Charo, J Werr, B Olsson, F Idali, L Lindbom, R Kiessling, H Jörnvall, H Wigzell, G H Gudmundsson

Stimulation of T and NK cells with IL-6, IFN-g enhances secretion of LL-37 in vitro

APOSEC
“Apoptotoc Secretome”

- Secretome of irradiated Blood Mononuclear Cells
  - lymphocytes (B cells (~15 %), T cells (~70 %)), natural killer cells (NK cells) (~10 %), monocytes (~5 %)
- Secreted from stressed MNCs striving to survive
  - Comprising: proteins: cytokines and growth factors, pro-angiogenic substances, lipids, free nucleic acids and extracellular vesicles: such as exosomes, microparticles

Sci Rep. 2015;16;5:16662



Apoptosis. 2016 Dec;21(12):1336-1353

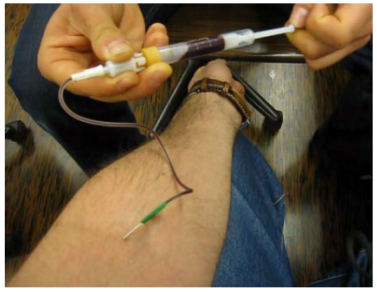
Eur J Clin Invest. 2016 Oct;46(10):853-63. doi: 10.1111/eci.12667. Epub 2016 Sep 26.

Dying Blood Mononuclear Cell Secretome Exerts Antimicrobial Activity

Mohammad Mahdi Kasiri [1], Lucian Beer [1 2], Lucas Nemec [3], Florian Gruber [4 5], Sabine Pietkiewicz [6], Thomas Haider [1], Elisabeth Maria Simader [1], Denise Traxler [1], Thomas Schweiger [3], Stefan Janik [1], Shahrokh Taghavi [3], Christian Gabriel [7], Michael Mildner [8], Hendrik Jan Ankersmit [9 10 11]

Methods
Prep. MNC sec.

Venous Blood Withdrawal



Cell Separation

Irradiation

Incubation for 24h

Centrifugation

Lyophilization

Lyophilized MNC-secretomes

Virus Elimination (GMP)

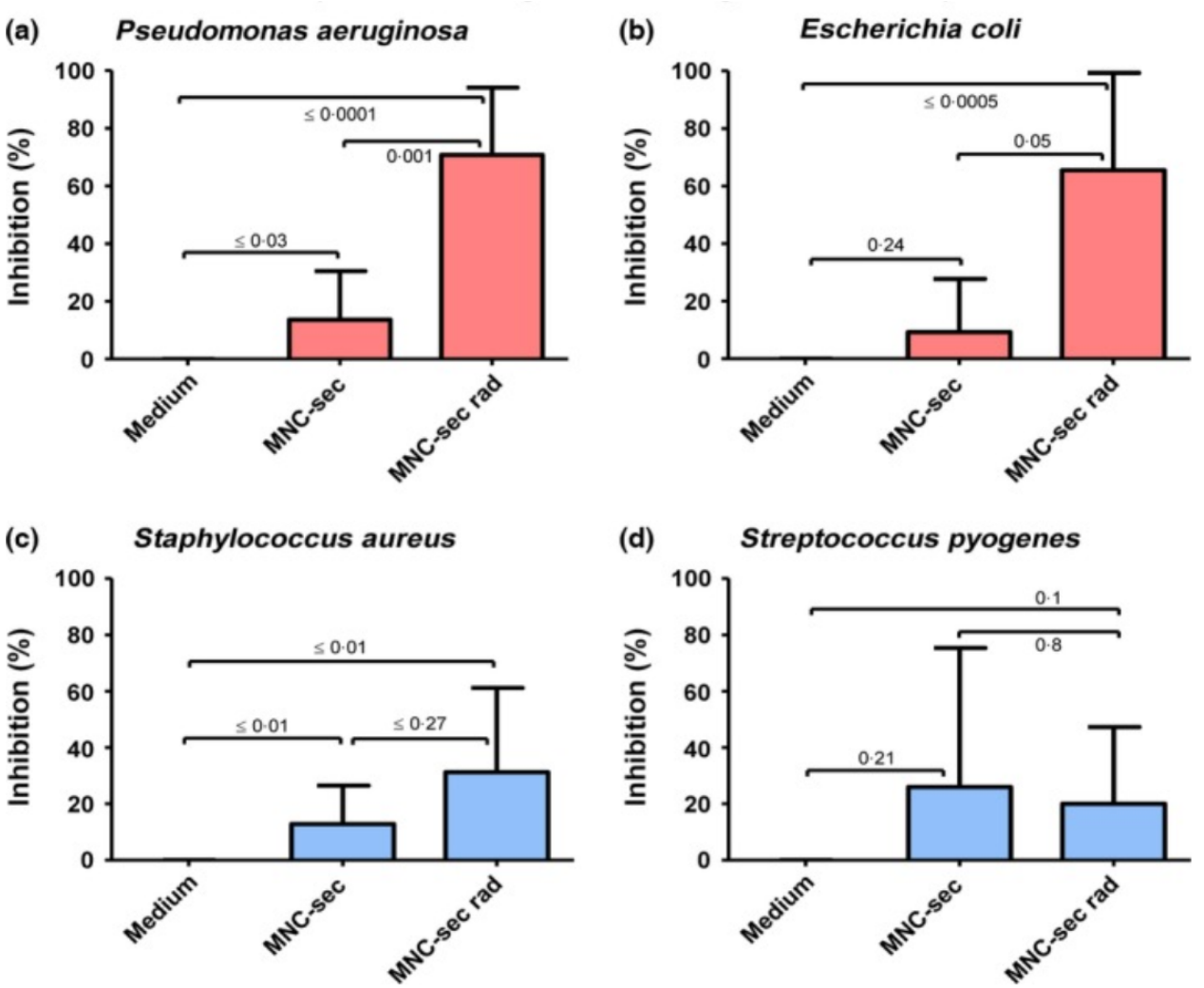
Methods

- Applied techniques:
  - Microdilution assay for antimicrobial activity
  - ELISA: AMPs
  - PCR: MNCs
  - In-vivo animal experiment with Sprague–Dawley rats
  - Flow Cytometry using Imaging Flow Cytometer for invest. of cell death

Eur J Clin Invest. 2016 Oct; 46(10): 853–863

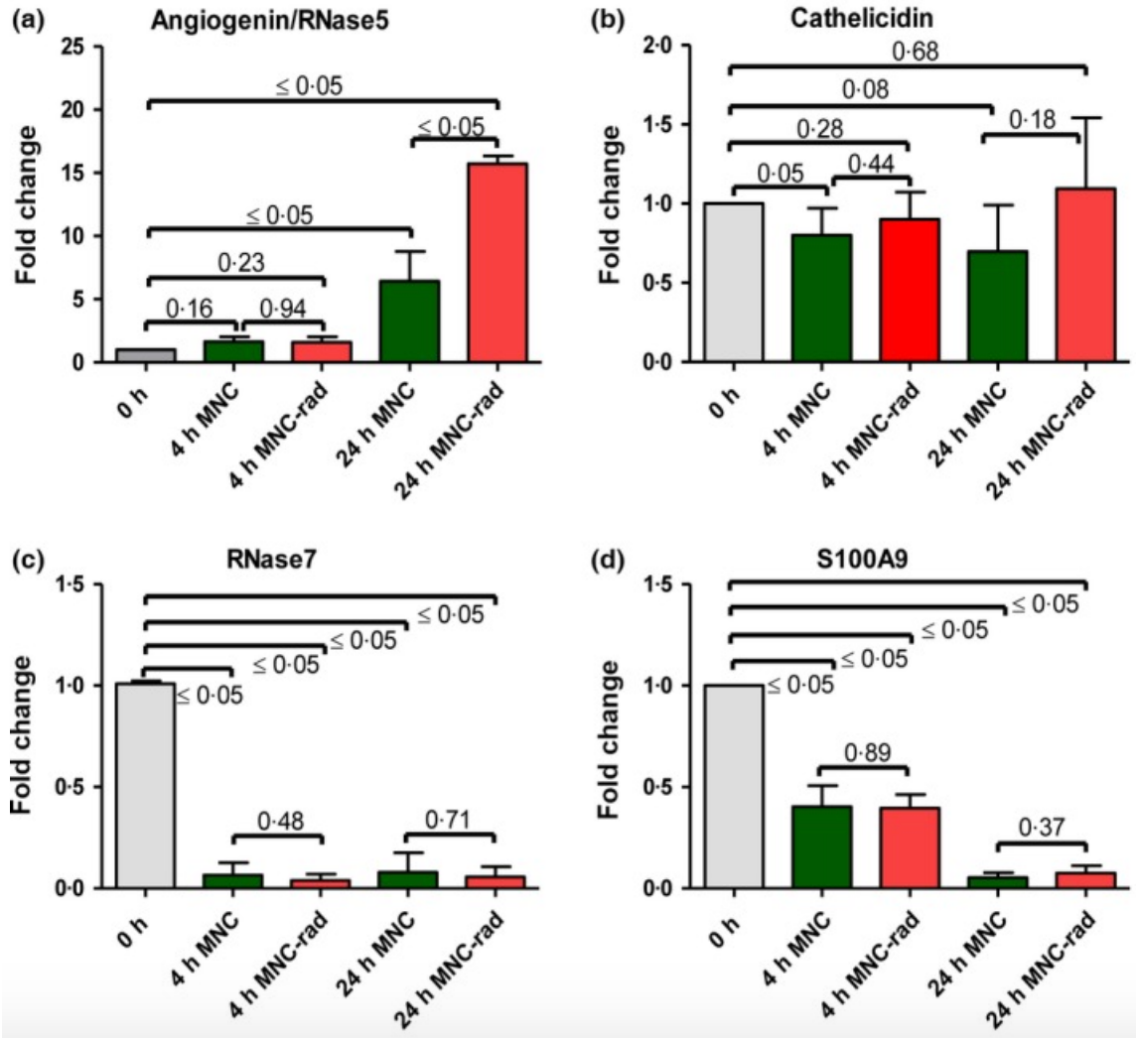
Results

Antimicrobial activity of MNC sec against G-&G+ bacteria



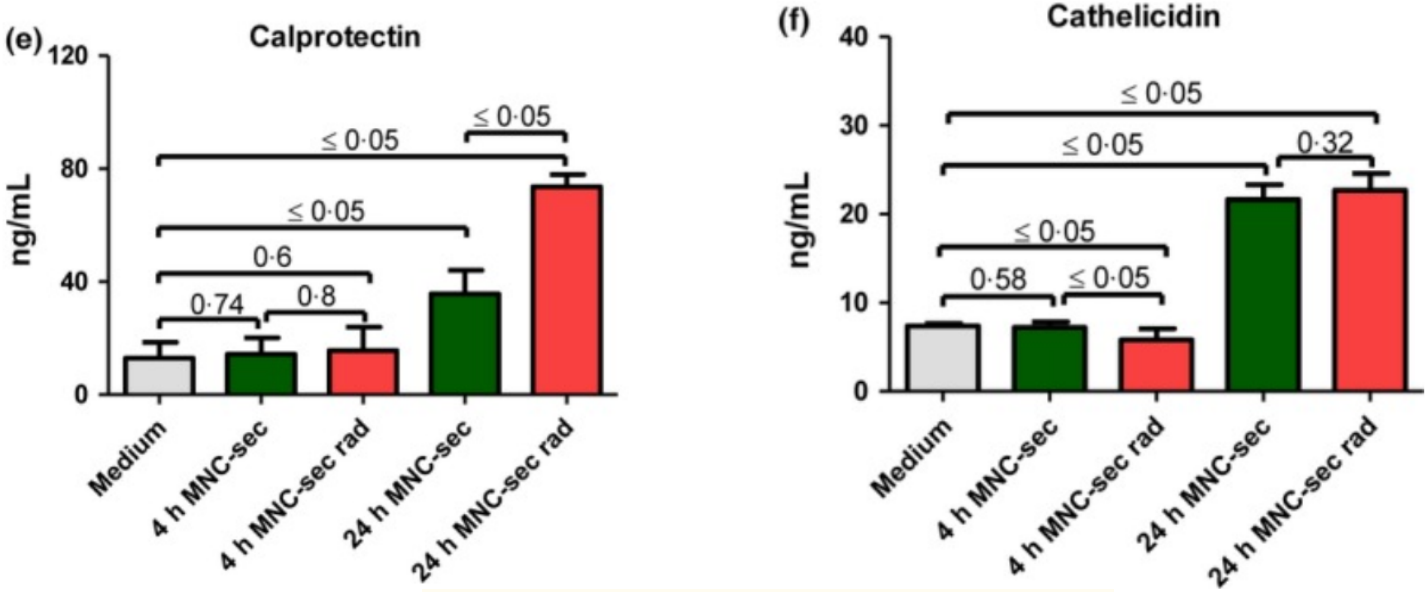
Eur J Clin Invest. 2016 Oct; 46(10): 853–863

Results
AMP expression in MNC-rad vs. MNC-non-rad



Eur J Clin Invest. 2016 Oct; 46(10): 853–863

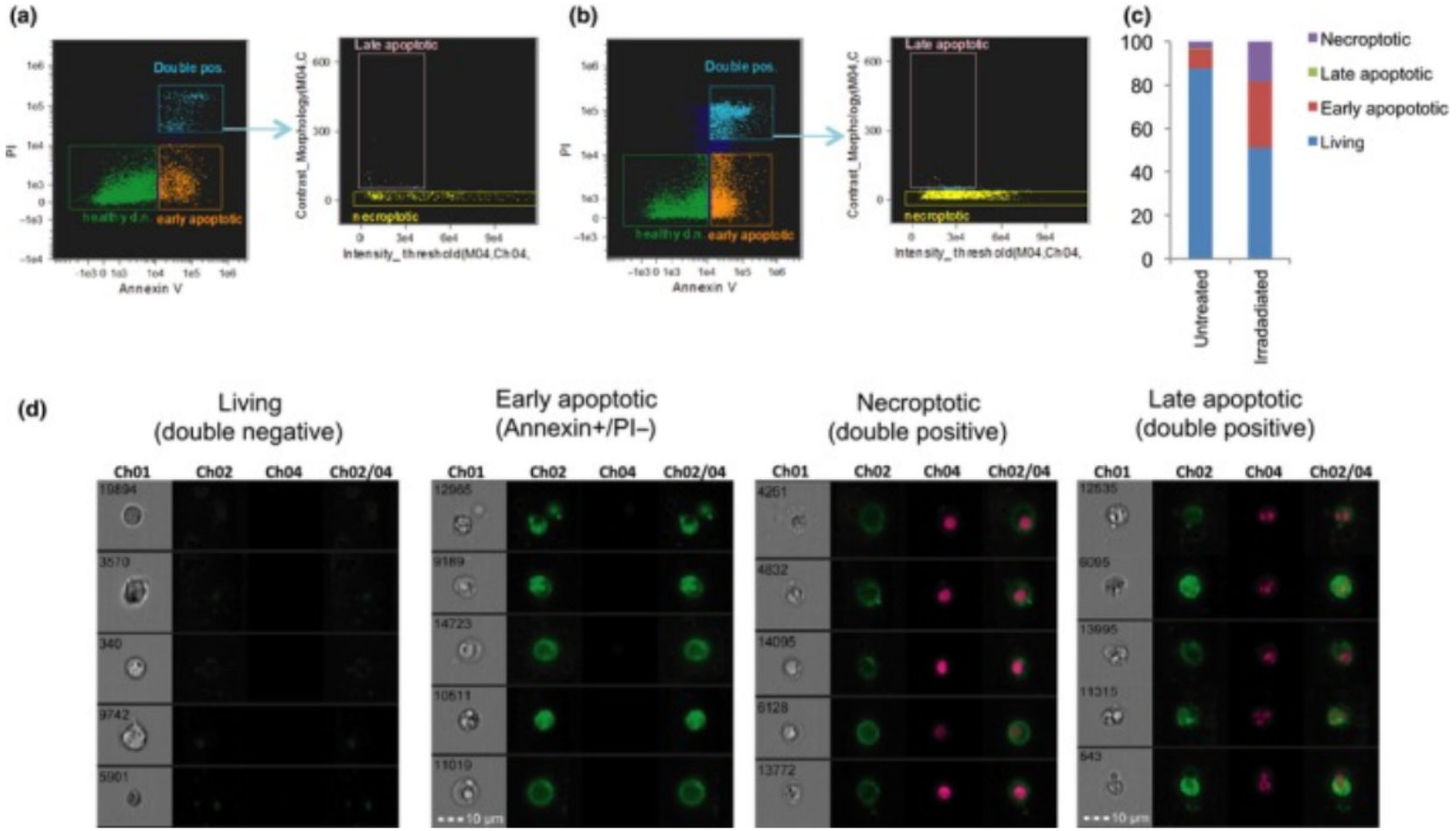
Results
AMP consentration in MNC-rad vs. MNC-non-rad



AMPs	GMP MNC-sec ng/ml
Cathelicidin	24·75 ± 5
Calprotectin	82·25 ± 4
RNase 3	19·75 ± 3
DEFB 124	2·75 ± 1

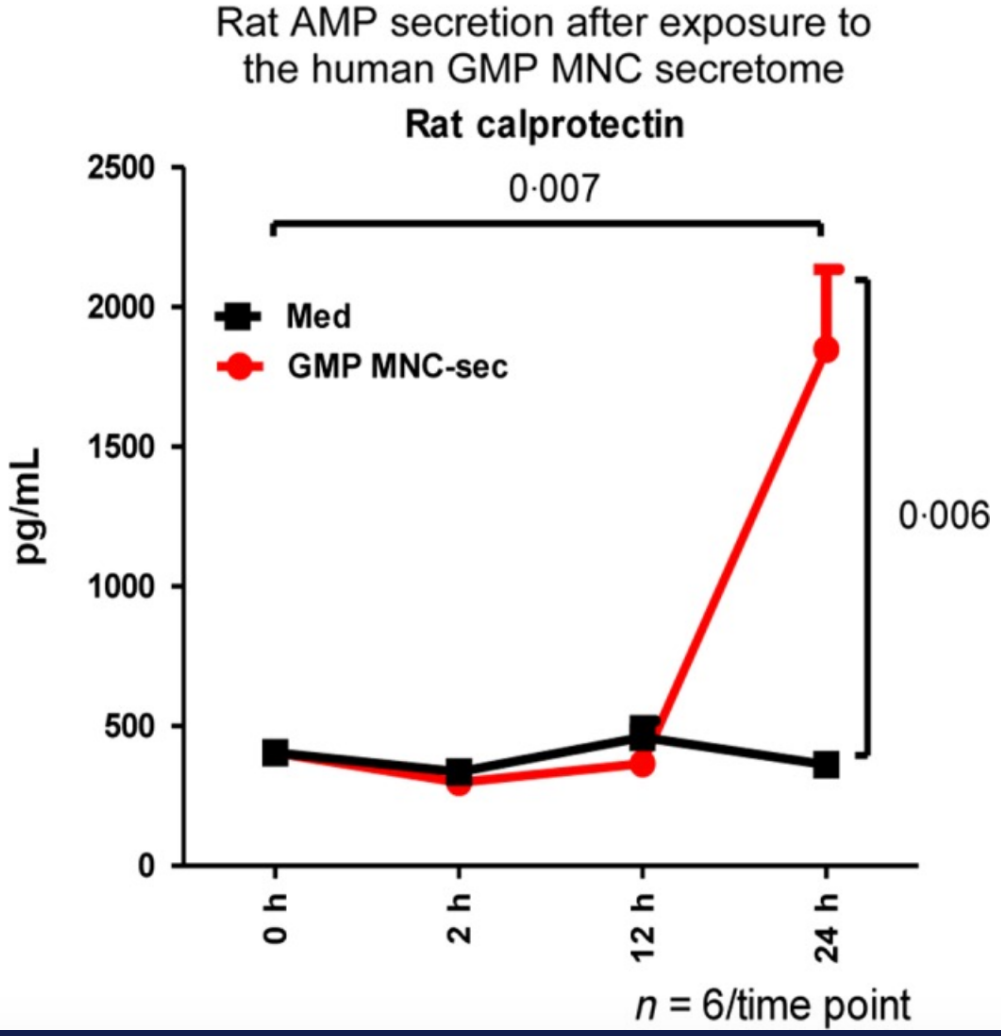
Eur J Clin Invest. 2016 Oct; 46(10): 853–863

Results
Mechanism of cell death – sec. phenotype



Eur J Clin Invest. 2016 Oct; 46(10): 853–863

Results
APOSEC timulates endogenous AMP production in rats



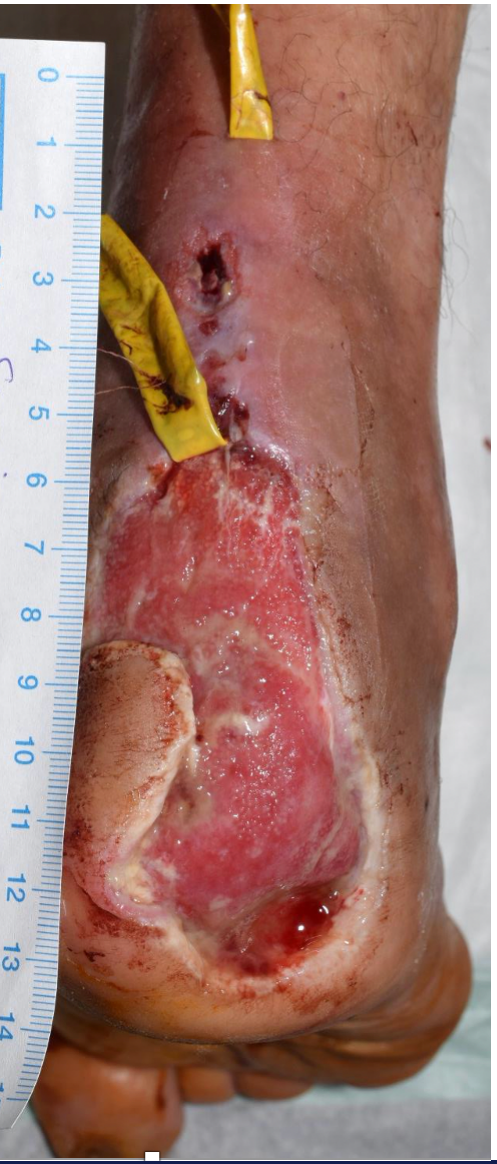
Eur J Clin Invest. 2016 Oct; 46(10): 853–863

APOSEC

- Irradiation → increased AMP production in MNCs
- Direct and indirect positive effects on immune system include
  1. Direct antibacterial activity
  2. Augmentation of the endogenous AMP production

- The advantages of APOSEC include
  1. easy access to the source cells
  2. acellular content of the product, preventing the risk of an unwanted cell dependent immune reaction
  3. Simple production

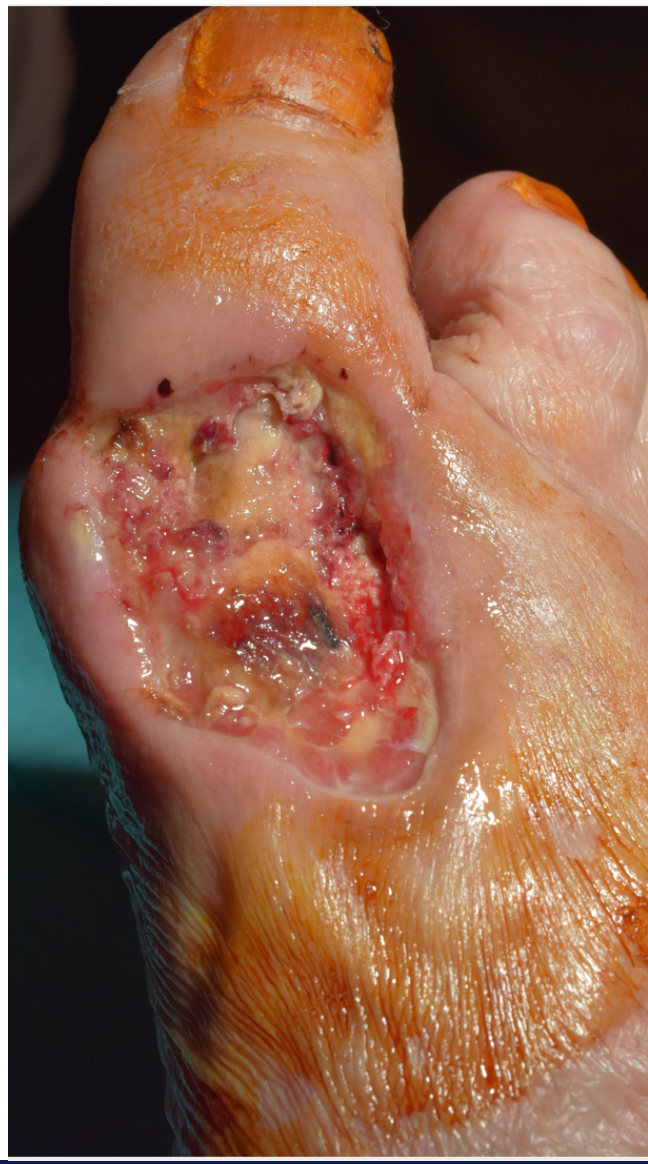
Diabetic Foot Ulcer (DFU)



- Chronic wound condition
- Reduced quality of life
- Physical, psychological, and economical burden
- Infection in 50% cases
- Limb amp. in 25% cases

Characteristics:

1. Impaired wound healing capacity
2. PNP
3. Vascular insufficiencies
4. Inflammation, infection



Fed Pract. 2016 Feb; 33(2): 16–23

APOSEC

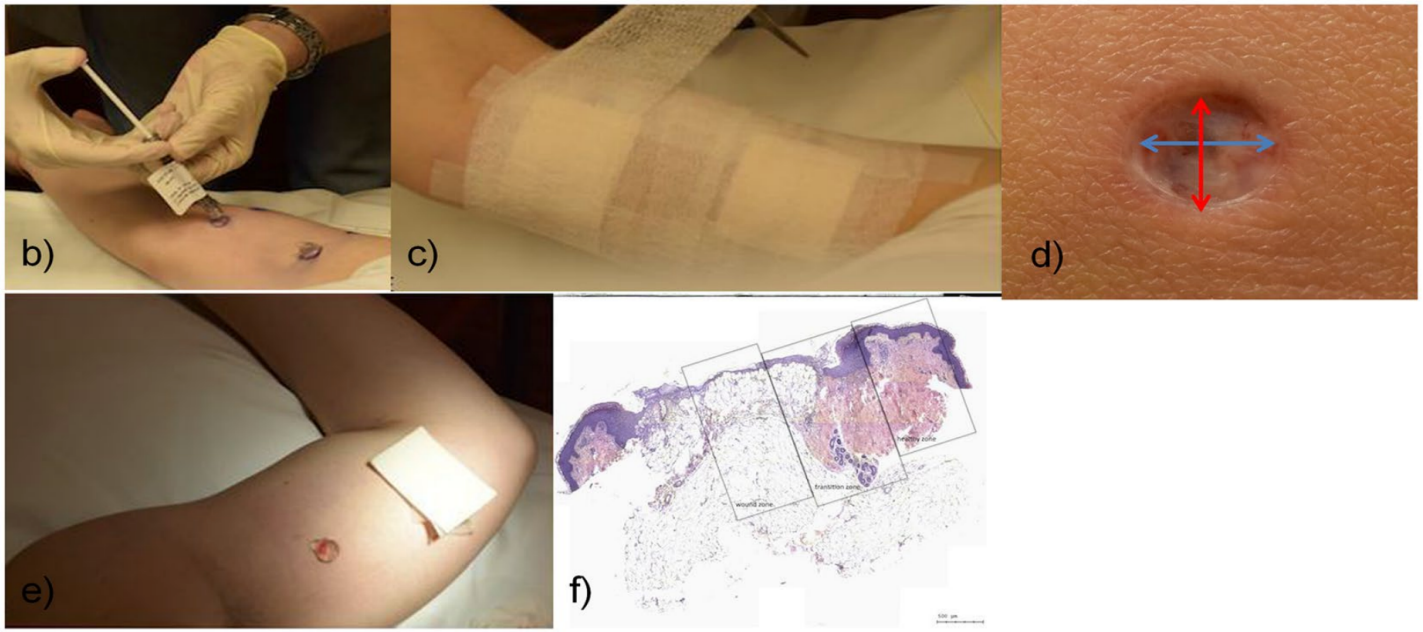
1. Inhibition of microvascular obstruction,
2. Vasodilation,
3. Angiogenesis,
4. Enhanced migration of human primary keratinocytes,
5. Neuroregeneration,
6. Improvement and acceleration of wound healing,
7. Antimicrobial and anti-inflammatory capacity,
8. Increasing expression of endogenous AMPs

Basic Res Cardiol. 2012;107(5):292
Eur Heart J. 2013;36(11):676-85
PLoS One. 2013;8(3)(3):e60103
Exp Neurol. 2015;S0014-4886(15):00081-3

Marsyas I

Clinical Trial > Sci Rep. 2017 Jul 24;7(1):6216. doi: 10.1038/s41598-017-06223-x.

Safety and Tolerability of Topically Administered Autologous, Apoptotic PBMC Secretome (APOSEC) in Dermal Wounds: A Randomized Phase 1 Trial (MARSYAS I)



Marsyas II

Christian Doppler Laboratory for Cardiac and Thoracic Diagnosis & Regeneration

NIH U.S. National Library of Medicine
ClinicalTrials.gov

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Home > Search Results > Study Record Detail

Save this study

Trial record **1 of 1** for: marsyas

Previous Study | Return to List | Next Study

A Study to Evaluate Safety and Efficacy of APO-2 at Three Different Doses in Patients With Diabetic Foot Ulcer

ClinicalTrials.gov Identifier: NCT04277598

Recruitment Status : Not yet recruiting
First Posted : February 20, 2020
Last Update Posted : February 24, 2020

See Contacts and Locations

Sponsor:
Aposcience AG

Collaborator:
FGK Clinical Research GmbH

Information provided by (Responsible Party):
Aposcience AG

DFU Named-Pat. Use

- Day 1 →

- Day 45 →