INSTITUTE OF MOLECULAR BIOMEDICINE

EXTRACELULÁRNA DNA U PACIENTOV S ALKOHOLOVOU CHOROBOU PEČENE

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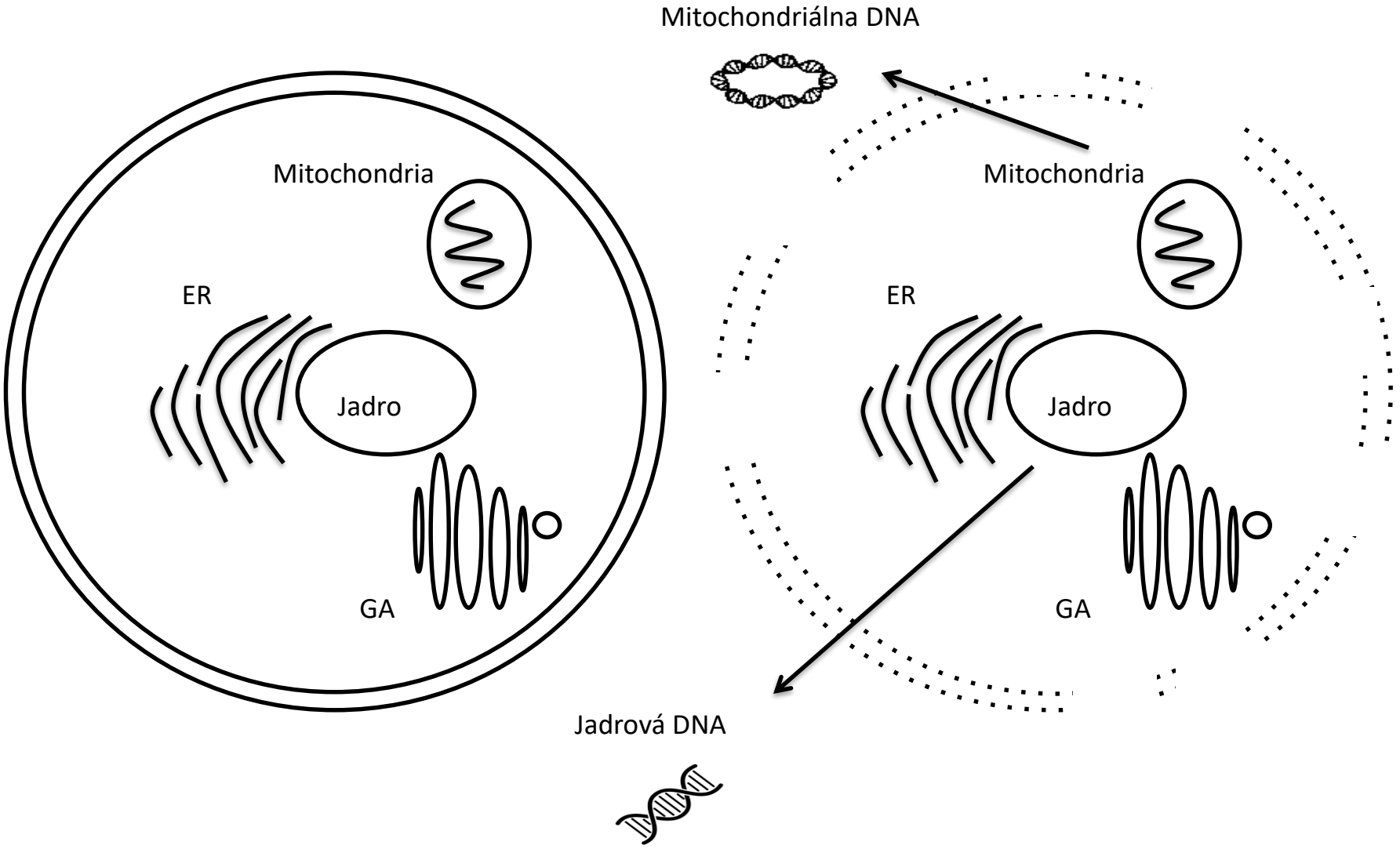
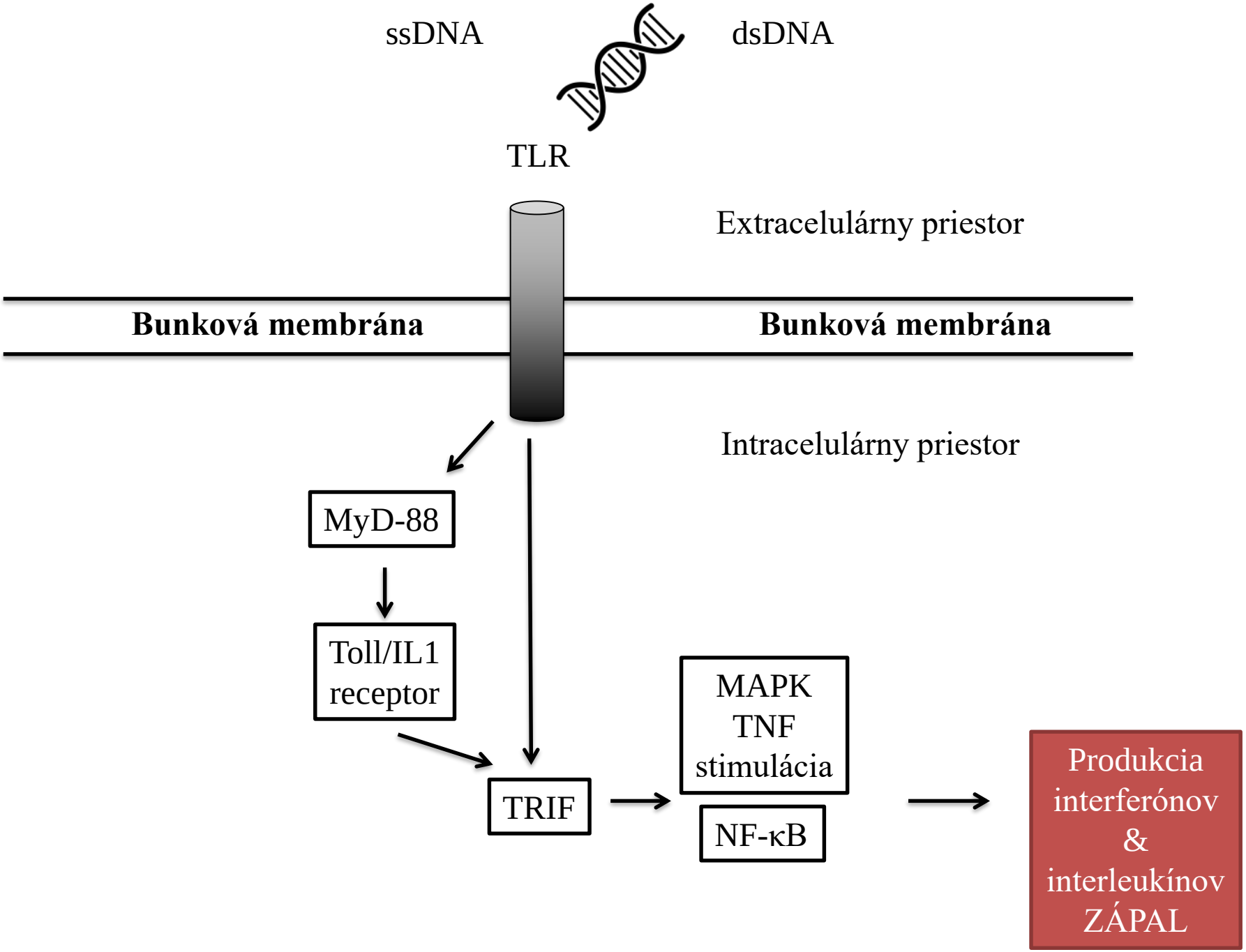


Extracelulárna DNA (ecDNA): jadrová (ncDNA), mitochondriálna (mtDNA)


ssDNA
dsDNA
TLR
Extracelulárny priestor
Bunková membrána
Bunková membrána
Intracelulárny priestor
MyD-88
Toll/IL1 receptor
TRIF
MAPK
TNF
stimulácia
NF-κB
Produkcia interferónov & interleukínov
ZÁPAL

Hepatopatie

- MtDNA pochádzajúca z pečene spôsobuje nealkoholovú steatohepatitídu

The Journal of Clinical Investigation BRIEF REPORT

Hepatocyte mitochondrial DNA drives nonalcoholic steatohepatitis by activation of TLR9

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Nonalcoholic steatohepatitis (NASH) is the most common liver disease in industrialized countries. NASH is a progressive disease that can lead to cirrhosis, cancer, and death, and there are currently no approved therapies. The development of NASH in animal models requires intact TLR9, but how the TLR9 pathway is activated in NASH is not clear. Our objectives in this study were to identify NASH-associated ligands for TLR9, establish the cellular requirement for TLR9, and evaluate the role of obesity-induced changes in TLR9 pathway activation. We demonstrated that plasma from mice and patients with NASH contains high levels of mitochondrial DNA (mtDNA) and intact mitochondria and has the ability to activate TLR9. Most of the plasma mtDNA was contained in microparticles (MPs) of hepatocyte origin, and removal of these MPs from plasma resulted in a substantial decrease in TLR9 activation capacity. In mice, NASH development in response to a high-fat diet required TLR9 on lysozyme-expressing cells, and a clinically applicable TLR9 antagonist blocked the development of NASH when given prophylactically and therapeutically. These data demonstrate that activation of the TLR9 pathway provides a link between the key metabolic and inflammatory phenotypes in NASH.

Introduction

Hepatocyte steatosis and ballooning and inflammation of the liver constitute steatohepatitis, which occurs in response to a diverse range of stressors ranging from excess alcohol to drug-induced liver injury. Obesity-driven metabolic syndrome is the most common reason for steatohepatitis and is termed nonalcoholic steatohepatitis (NASH) (1). Overnutrition and obesity uniformly result in hepatic steatosis, which can progress to steatohepatitis, fibrosis, and cirrhosis. The biological processes promoting progression from steatosis to steatohepatitis are of great interest but are currently poorly understood.

TLR9 is an endosomal pattern recognition receptor (PRR) for which CpG-rich bacterial DNA and mammalian self-DNA are ligands (2). We have identified a requirement for TLR9 in toxic liver inflammation and fibrosis (3, 4), and this has been confirmed for hepatic steatosis and inflammation in the choline-deficient amino acid–defined diet model of NASH (5). Apart from this basic finding of a requirement for TLR9 in the development of NASH, very little is known about the identity of TLR9 ligands and how the TLR9 pathway is activated in NASH. The metabolic changes in NASH suggest that there may be unique interactions with TLR9 biology in NASH, beyond that found for liver injury in general. For example, mitochondrial DNA (mtDNA) has many features that make it a potent ligand for TLR9, and there is an increase in hepatocyte mitochondrial content in NASH (6).

The demonstration of an increase in total liver mtDNA and oxidized DNA (oxDNA) in NASH and the ability of these molecules to activate TLR9 suggest a potential mechanism by which the changes induced by overnutrition result in greater activation of a proinflammatory pathway that leads to a transition from steatosis to steatohepatitis (6, 7).

Results and Discussion

We tested whether hepatocyte nuclear DNA (nDNA) and mtDNA from high-fat diet–induced (HFD-induced) NASH livers are more potent activators of TLR9 than DNA from control livers. Figure 1A shows that mtDNA isolated from hepatocytes of mice fed a HFD for 12 weeks resulted in greater activation of a TLR9 reporter than did mtDNA from mice fed a chow diet (CD). This was confirmed in mouse Kupffer cells (KCs), which demonstrate upregulation of *Tnfa* transcripts in response to hepatocyte mtDNA from HFD-fed mice (Figure 1B).

Hepatocyte mtDNA is increased in NASH and is known to be proinflammatory (6). For hepatocyte mtDNA to activate TLR9, it would require release from hepatocytes and entry into TLR9–containing endosomes of cells capable of mounting an inflammatory response. Plasma extracellular mtDNA levels increase in tissue injuries in mice and humans including toxic liver injury (8). We tested whether a 12-week HFD model of NASH resulted in an increase in plasma DNA. There was an increase in total DNA and mtDNA, but not nDNA in plasma from HFD-fed compared with CD-fed mice (Supplemental Figure 1, A–C; supplemental material available online with this article; doi:10.1172/JCI83885DS1). Plasma from HFD-fed mice gave a small but significantly greater signal than did plasma from CD-fed mice from a TLR9 reporter cell line (Supplemental Figure 1D). To confirm whether these changes were present in humans, plasma from 3 previously well–characterized groups was analyzed: group 1 consisted of lean subjects without

Conflict of interest: R.L. Coffman and A. Candia are employees of Dynavax Technologies.
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Intrahepatálna cholestáza

- Zvýšená ecDNA → ďalšie poškodenie pečene

Prenatal Diagnosis *Explore this journal >*

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Research Letter

Cell-free DNA is higher and more fragmented in intrahepatic cholestasis of pregnancy†

Barbora Vlková, Marta Kalousová, Anna Germanová, Antonín Pařízek, Zdeněk Hájek, Tomáš Zima, Peter Celec

Hepatorenálne poškodenie

- Podanie deoxyribonukleázy znižuje ecDNA počas hepatorenálneho poškodenia u potkanov

RESEARCH ARTICLE | *Liver and Biliary Tract Physiology/Pathophysiology*

Deoxyribonuclease partially ameliorates thioacetamide-induced hepatorenal injury

Lenka Vokálová,[1] Lucia Lauková,[2] Jozef Čonka,[2] Veronika Melišková,[2] Veronika Borbélyová,[2] Janka Bábíčková,[2] L'ubomíra Tóthová,[2] Július Hodosy,[1,2] Barbora Vlková,[2] and Peter Celec[2,3,4]

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Alkoholová choroba pečene

- Zomretí
  - n = 15

- Preživší
  - n = 18

EcDNA pri alkoholovej chorobe pečene – potencionálny marker

Model For End-Stage Liver Disease (MELD) for ages 12 and older

The Model for End Stage Liver Disease (MELD) predicts survival for patients with advanced liver disease.

The United Network for Organ Shar
system on January 11, 2016 that is r
determining the traditional MELD s
score is adjusted by incorporating t

MELD

Serum Bilirubin *(mg/dL)*:

INR (International Normalized Ra

Serum Creatinine *(mg/dL)*:

Did the patient have dialysis at le
prior week?

Serum Sodium *(mmol/L)*:

CLIF-C ACLF (Acute-on-Chronic Liver Failure) score and expected mortality rates

ACLF Grade, CLIF-C ACLF Score (ACLF patients) and CLIF-C AD Score (non-ACLF patients with Acute Decompensation)

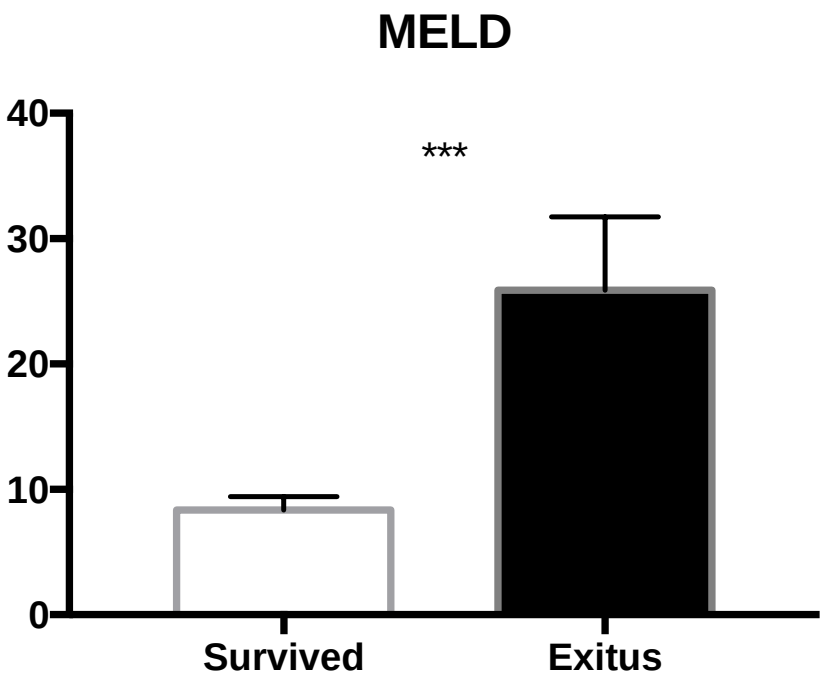
See score formula

	DATA	SCORES
Bilirubin	☐ mg/dl	Liver score ☐ Liver failure ○ Yes ○ No
Creatinine	☐ mg/dl	Kidney score ☐
Renal replacement therapy	○ Yes ○ No	Renal failure ○ Yes ○ No
Use of vasopressors (Hepatorenal syndrome indication)	○ Yes ○ No	
West-Haven grade for HE	○ 0 ○ 1 ○ 2 ○ 3 ○ 4	Brain score ☐ Cerebral failure ○ Yes ○ No
INR	☐	Coagulation score ☐ Coagulation failure ○ Yes ○ No
MAP	☐ mm/Hg	Circulation score ☐
Use of vasopressors (Circulatory failure indication)	○ Yes ○ No	Circulation failure ○ Yes ○ No
Select one ● PaO_2 (preferred) ○ SpO_2	☐	Lung score ☐

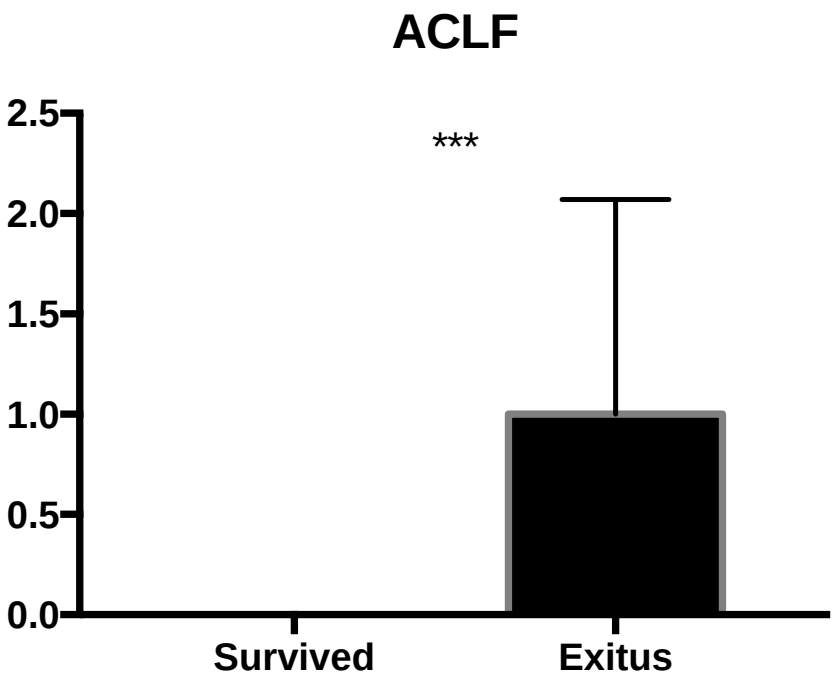
Metodika

- ecDNA v plazme
- ecDNA
  - ncDNA
  - mtDNA
  - bakteriálna DNA

Výsledky



p<0,0001



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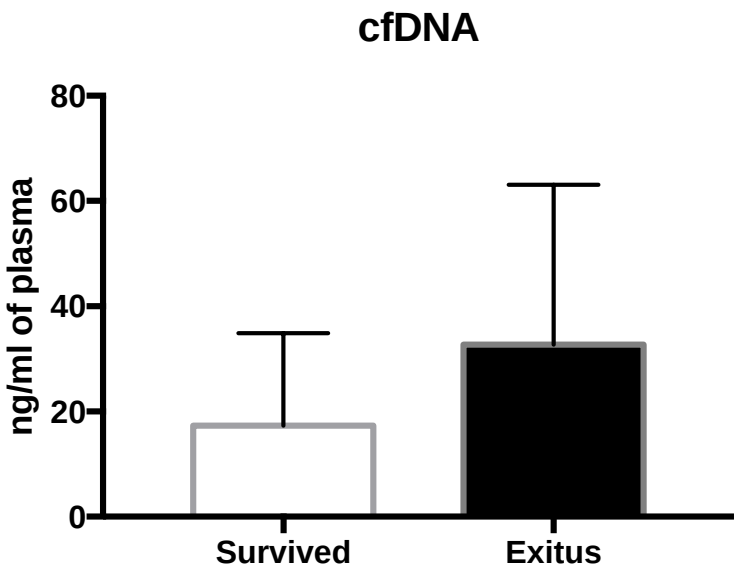
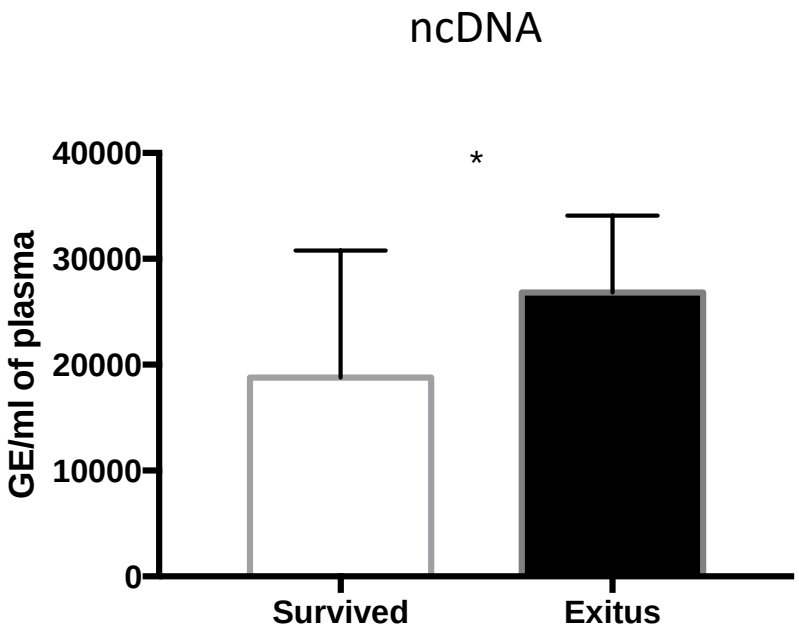
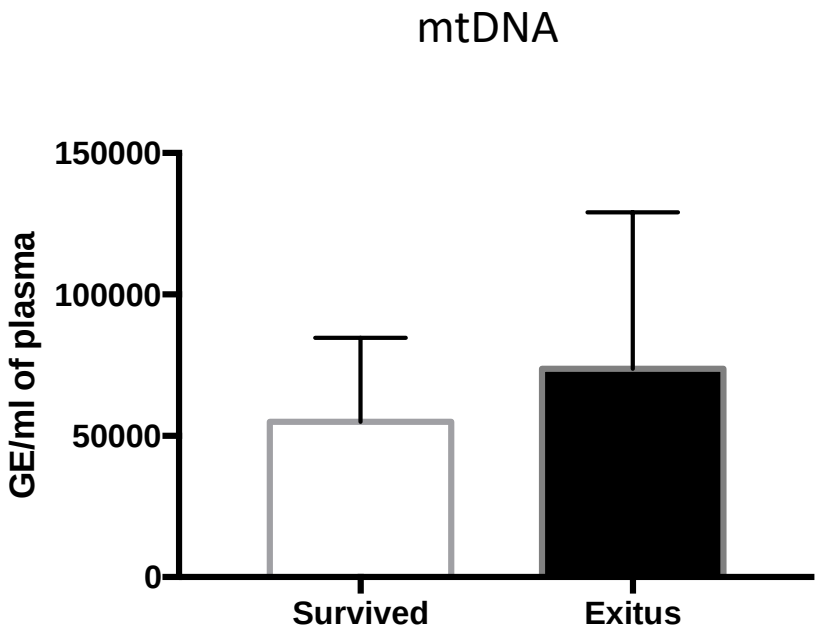
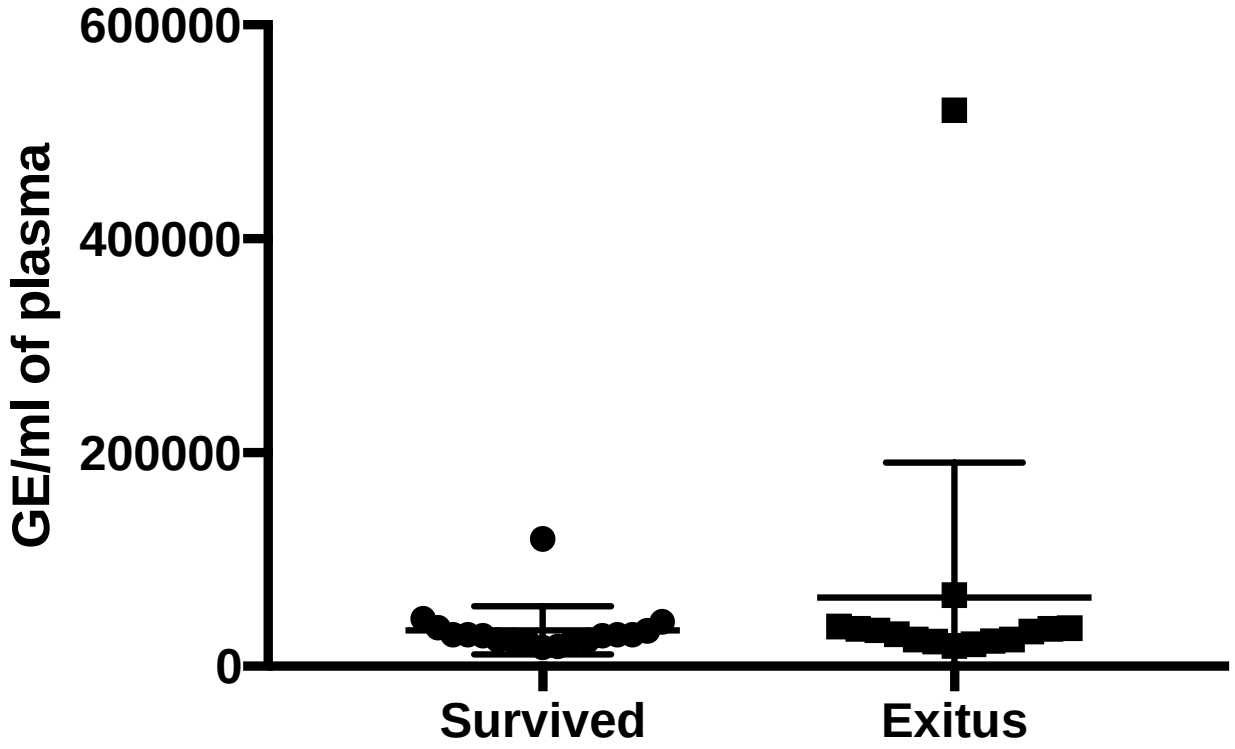

cfDNA
ng/ml of plasma
80
60
40
20
0
Survived
Exitus


ncDNA
GE/ml of plasma
40000
30000
20000
10000
0
*
Survived
Exitus


mtDNA
GE/ml of plasma
150000
100000
50000
0
Survived
Exitus

Bakteriálna extracelulárna DNA



Zhrnutie

- Klinické parametre – zhoršené pri zomretých

Zhrnutie

- Klinické parametre – zhoršené pri zomretých
- NcDNA – zvýšená pri zomretých

Záver

- Stav pacientov súvisí s ecDNA
- Heterogenita pacientov, malý počet pacientov → vplyv na výsledky

Poďakovanie

- MUDr. Ľubomír Skladaný, PhD.
- RNDr. Barbora Vlková, PhD.
- Doc. MUDr. Peter Celec, DrSc., MPH