In Vivo Imaging of Cell Death

7th Annual Training Course on Concepts and Methods in Programmed Cell Death

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Clinical Imaging Modalities for the Measurement of Apoptosis

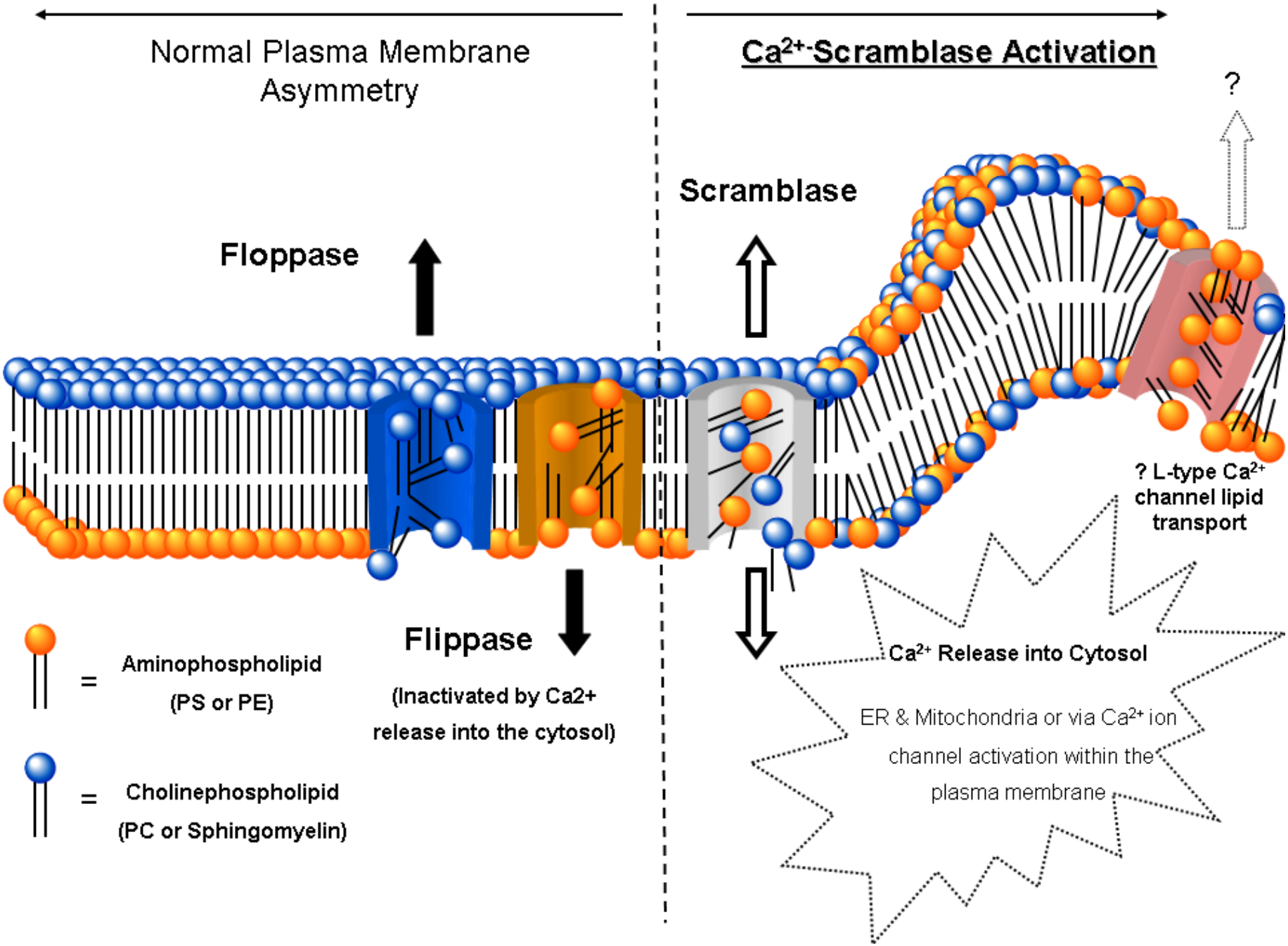
- Annexin V (Annexin V-128) / PS binding agents
- Choline & Methylene/Methyl Lipid Proton Water Suppressed MR Spectroscopy
- ML-9 & ML-10? / GE (no published data)? Mechanism? Scramblase?
- No other Methods Tested in Humans

Imaging Apoptosis with Annexin

- Annexin V: endogenous human protein (36kD, 319 amino acids)
- Plasma concentration 2 ng/mL
- Binds with antibody-like (nanomolar) affinity to membrane bound phosphatidylserine (PS)
- PS is only exposed to circulating annexin at surface of stressed cells
  - –PS externalized early in apoptotic cascade
  - –PS accessible on inner leaflet of necrotic cells due to permeability

Annexin V

- 0 to 200 PS binding sites/cell on all viable cell types (exception: endothelial cells with about 50,000 sites/cell at rest)
- Number of PS binding sites per cell = 10^5 to 10^6 with apoptosis
- One annexin V binds up to 8 PS anionic head groups
- Annexin binding is associated with all types of cell death (necrosis/oncosis, mitotic catastrophe, cell senescence, pyroptosis, PARP-1 mediated cell death and autophagy).


Normal Plasma Membrane Asymmetry
Ca2+-Scramblase Activation
?
Scramblase
Floppase
? L-type Ca2+ channel lipid transport
Flippase
(Inactivated by Ca2+ release into the cytosol)
Ca2+ Release into Cytosol
ER & Mitochondria or via Ca2+ ion channel activation within the plasma membrane
= Aminophospholipid (PS or PE)
= Cholinephospholipid (PC or Sphingomyelin)

Labeling of Annexin V Mutants via Endogenous Chelation Site

Ala-Gly-Gly-Cys-Gly-His-Annexin V-128.

+ Technetium-99m, $SnCl_2$, glucoheptonate

⇓ 37° x 60 min

^{99m}Tc-Annexin V

(~100 µCi/µg, >90% RCY, >95% RCP)

- No exogenous chelate needed
- Order of magnitude less kidney/bone marrow uptake than Hynic-Annexin allows for superior imaging

Tait et al., Bioconjugate Chem 2000; 11:918

Annexin V Imaging for the Clinic

Self-chelating Annexin V-128 is the structure of choice as random chelation with bifunctional moieties (i.e. HYNIC-annexin V) significantly degrades in vitro and in vivo binding to PS.

Phase I and II trials with GMP material planned for Spring of 2011 in patients with RA. Sponsored by Atreus Pharmaceuticals (Ottawa, Canada) in partnership with AAA (Advanced Accelerator Applications, Saint Genis Pouilly, France).

? Annexin V F-18 with site specific labeling

Early Diagnosis would allow for timely deployment of effective drug therapy

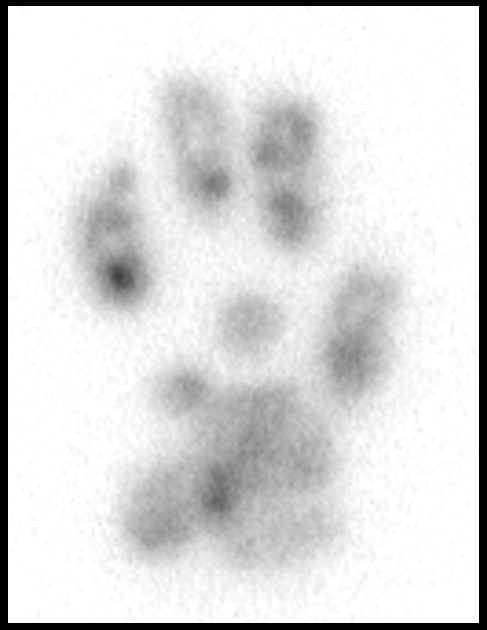
Early diagnosis of RA is essential to permit aggressive therapy with disease modifying drugs before loss of function

- Conventional radiography is an insensitive means of measuring changes in RA (1)
- Once joint destruction occurs, full function can never be restored
  - Current radiographic techniques provide detail of bone erosion and joint space narrowing “but only after clinical symptoms have been present for several months or even years.”(2)

(1) Taylor, P.C. “The value of sensitive imaging modalities in rheumatoid arthritis,” Arthritis Res. Ther., 5:210-13 (2003)

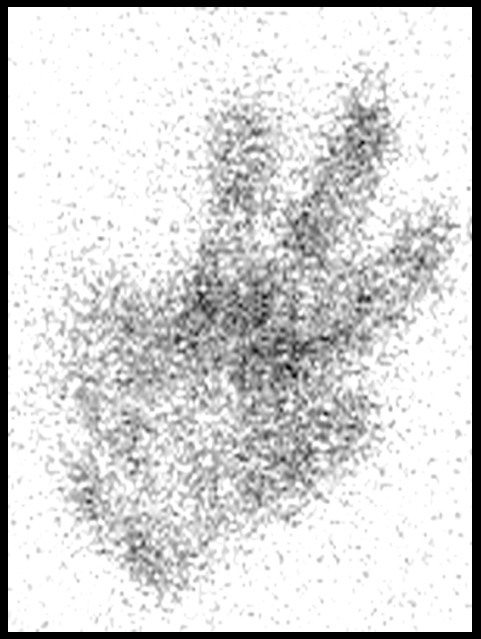
(2) S. Biswal, D.L.Resnick, J. M. Hoffman and S. Gambhir, “Integration of Molecular Imaging in to Musculoskeletal Imaging Practice,” Radiology 244:651-71 (2007)

Polyarticular Autoimmune Mediated Arthritis



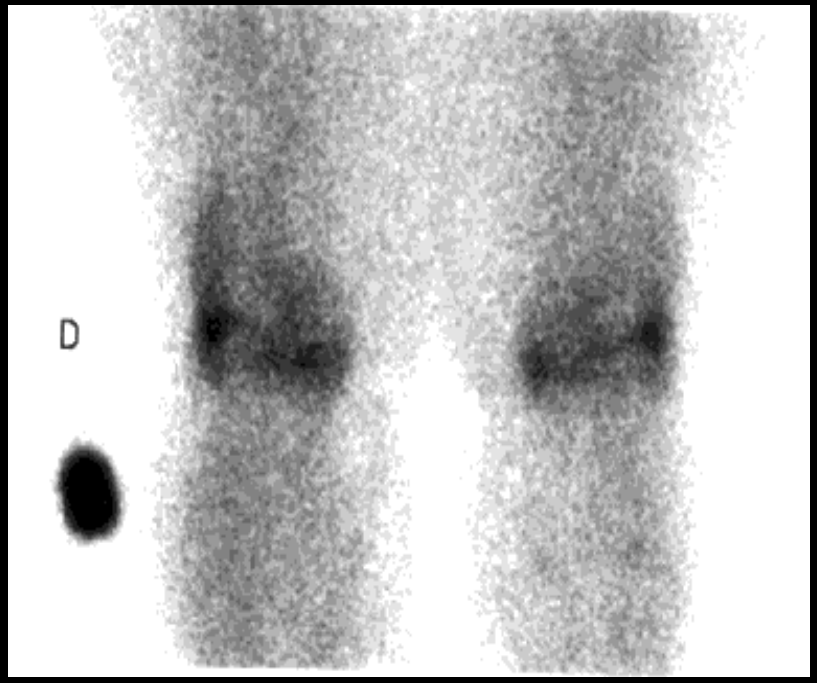
CONTROL

ARTHRITIS



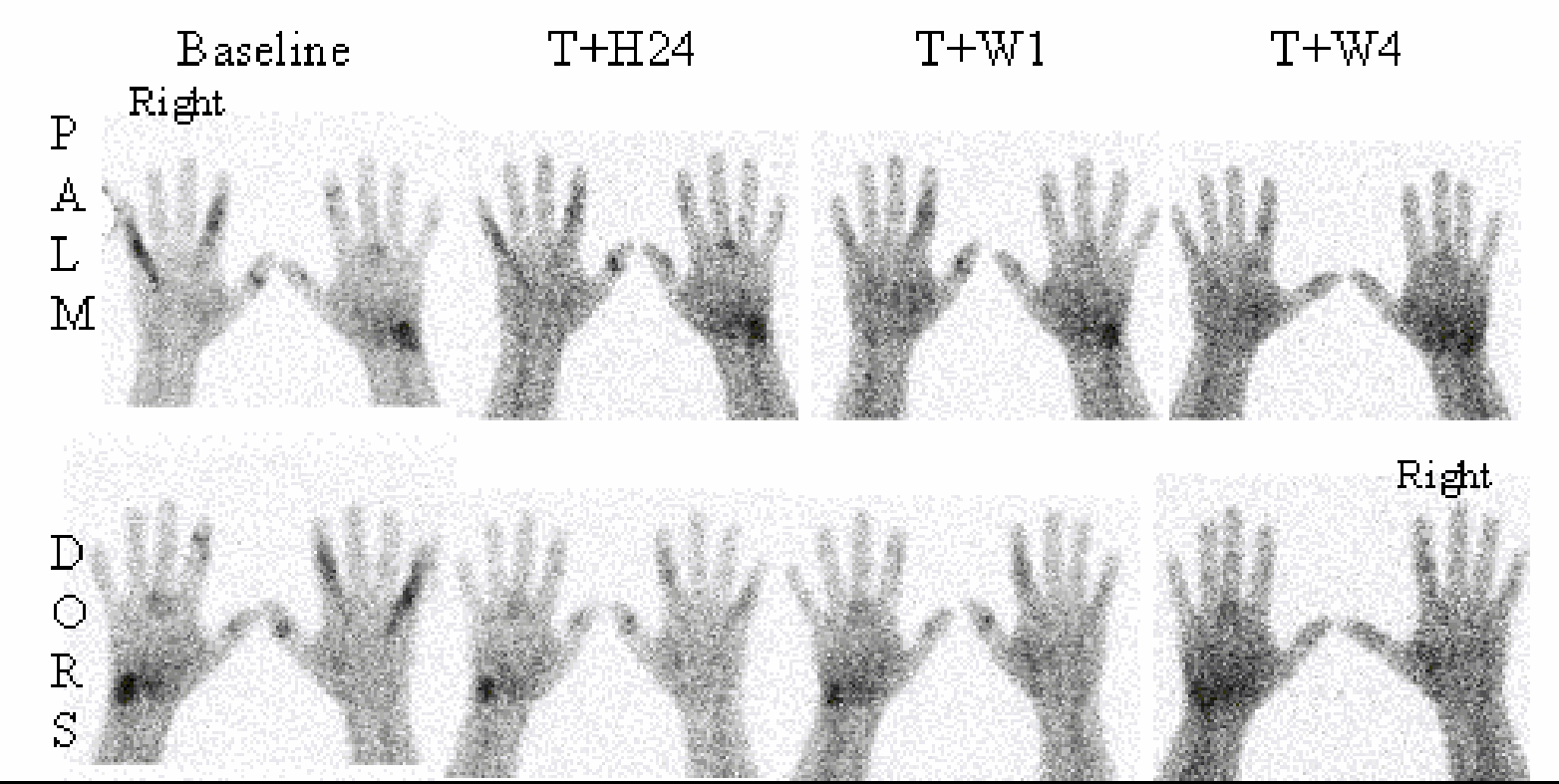
PREDNISONE

Tc-99m Annexin Imaging of RA in Humans



Spot views of Knees and hands in patient with RA using Tc-99m-hynic-annexin. Note marked synovial uptake in knees (and uptake in the tensor fascia lata of the right knee) as well as the carpal uptake in the right wrist

Response to RA Therapy



Serial spot views of RA Patient with Acute Flare before and after treatment with Chimeric anti-TNF monoclonal antibody (Infliximab™). Note tenosynovitis at baseline which resolves.

Annexin V Imaging

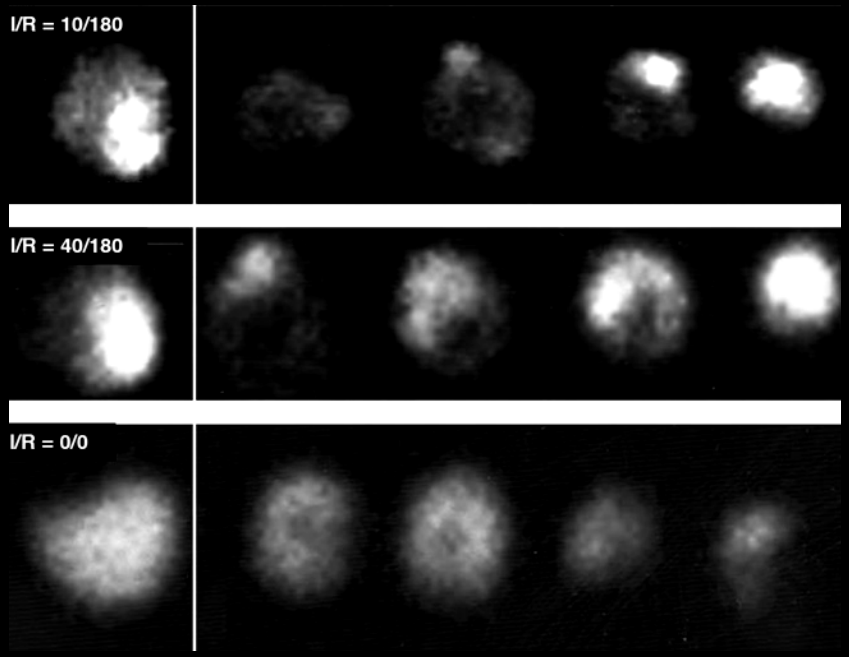
PS can be expressed at low levels in a reversible fashion under condition of cell stress that does not necessarily commit a cell to apoptotic cell death. (transient myocardial of cerebral ischemia, *RA / IBD (i.e. non-infectious inflammation, Infectious Inflammation*)

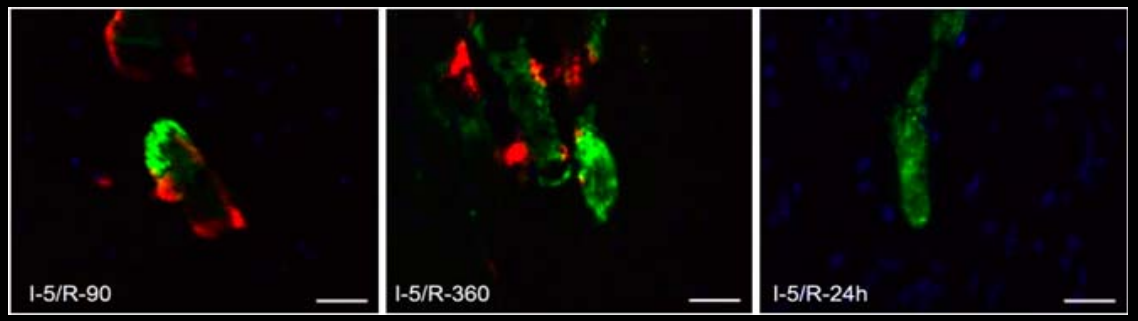
Annexin imaging can therefore define territory at risk and potentially salvageable with prompt intervention.

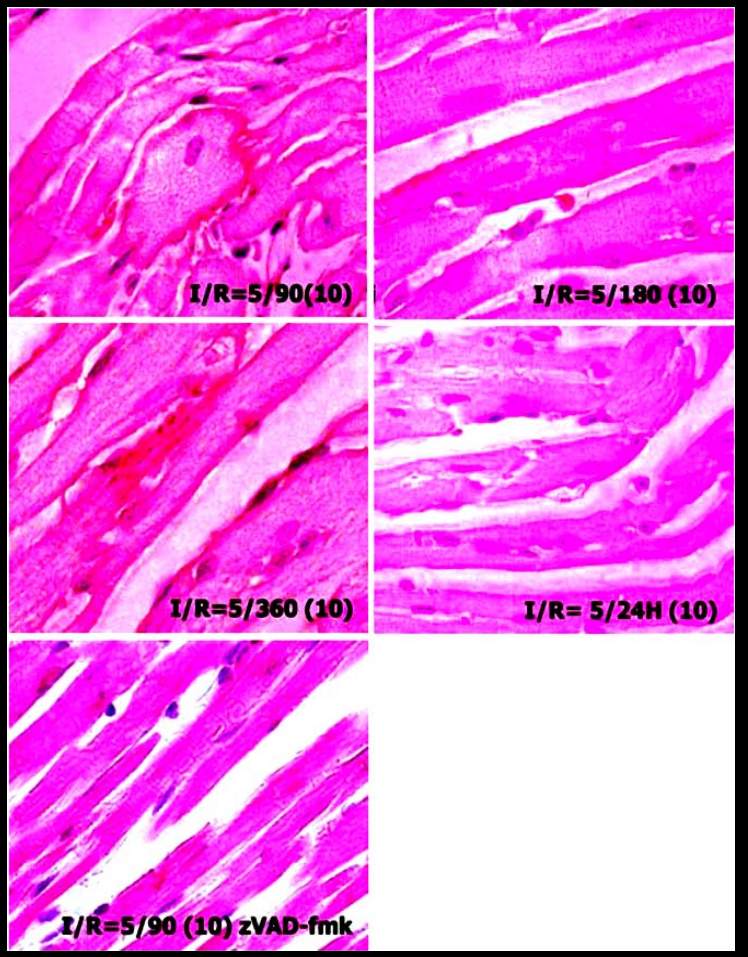
Reversible PS Expression in Myocardial Ischemia

- "Annexin A5 Uptake in Ischemic Myocardium: Demonstration of Reversible Phosphatidylserine Externalization and Feasibility of Radionuclide Imaging." Kenis H, et al. *J Nucl Med* 2010; 51:259–267.
- Short periods of ischemia of the LAD (5 & 10 mintues) there was a 9 fold increase in PS binding of annexin V that persisted for 6 hours after reperfusion in both rabbit and murine models. This 9 fold increase was approximately half of that observed with infarction (30 minutes of ischemia).
- Regions of transient ischemia demostrated low levels of caspase-3 activation and PS expression without evidence of TUNEL +.

Reversible PS Expression in Myocardial Ischemia (continued)







Caspase -3 Staining

Reversible PS Expression in Myocardial Ischemia (continued)

- Annexin V Imaging can be used as a marker of cellular stress and tissues that maybe salvageable with prompt intervention.
- Annexin V Imaging is therefore far more sensitive than potentially “specific” markers of apoptosis or necrosis (i.e. caspase-3, PARP-1, or markers of scramblase activity, ML-9).
- Annexin V binding and internalization via PS exposure maybe a way to selectively deliver anti-apoptotic drugs do regions of ischemic disease even after prompt reperfusion.

Hypoxic Ischemic Injury in a Pretem Infant

Coronal View: 1st day of life

Normal ?

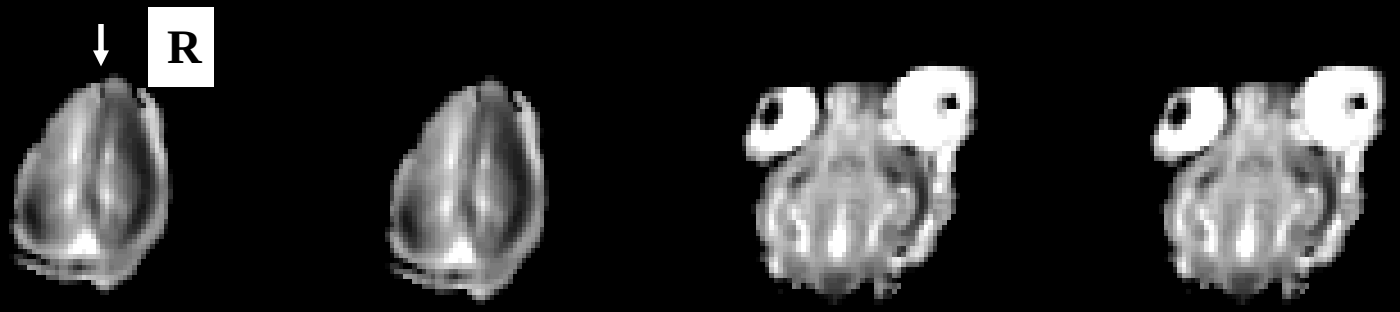
Sagittal View: day 21

Coronal View: day 21

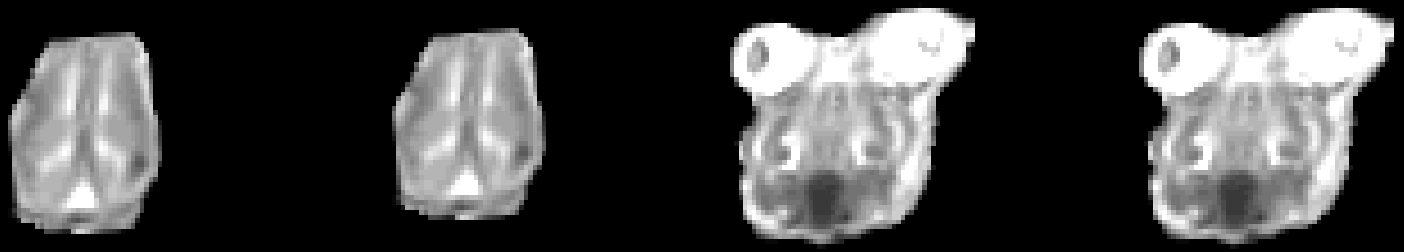
PVL

Neonatal Rabbit Model of Hypoxic-Ischemic Injury

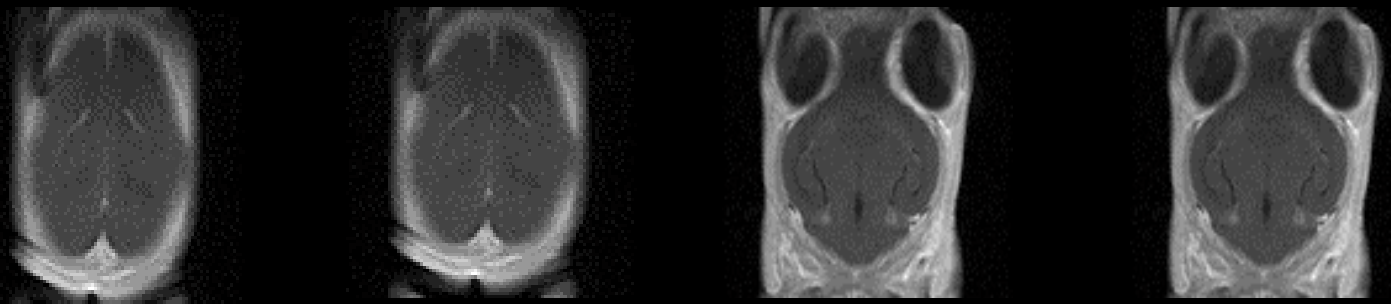
ADC Map after 60 mins hypoxia



ADC Map after recovery



T_1 W post Gd immediately prior to annexin V imaging



D'Arceuil et al. *Stroke* 2000

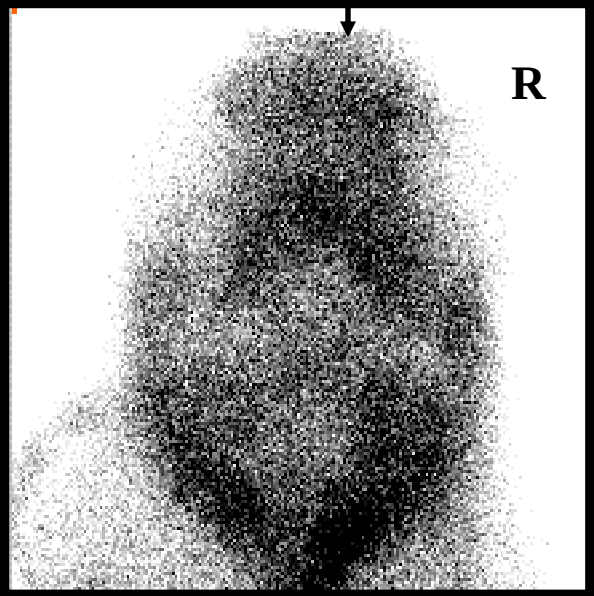
Two Hours After Hypoxia

In vivo

Ex vivo

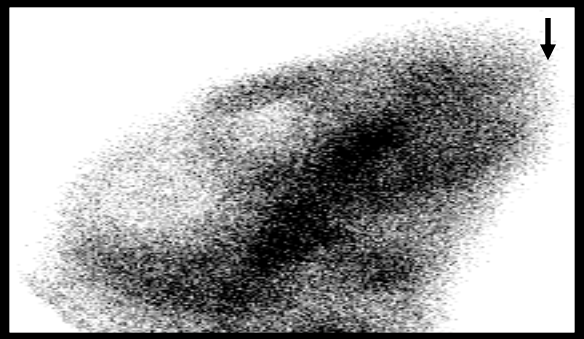
(A)

Post

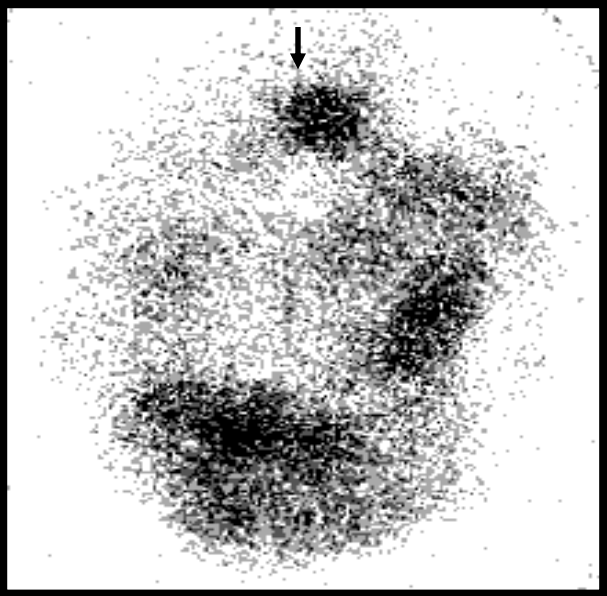


(B)

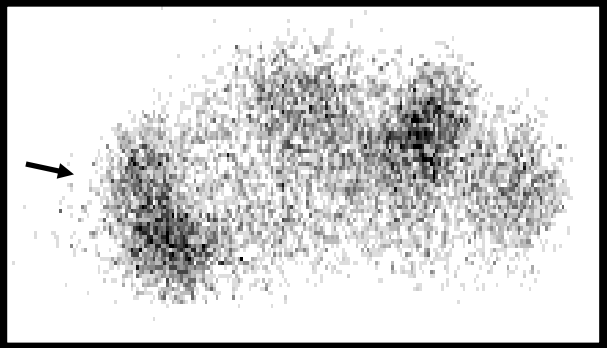
R Lat



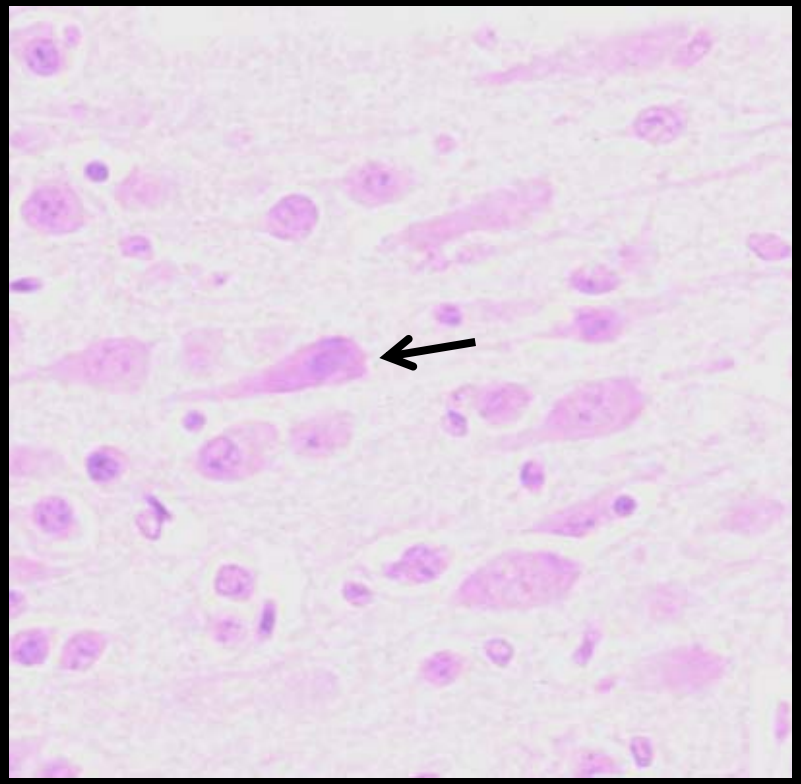
(C)



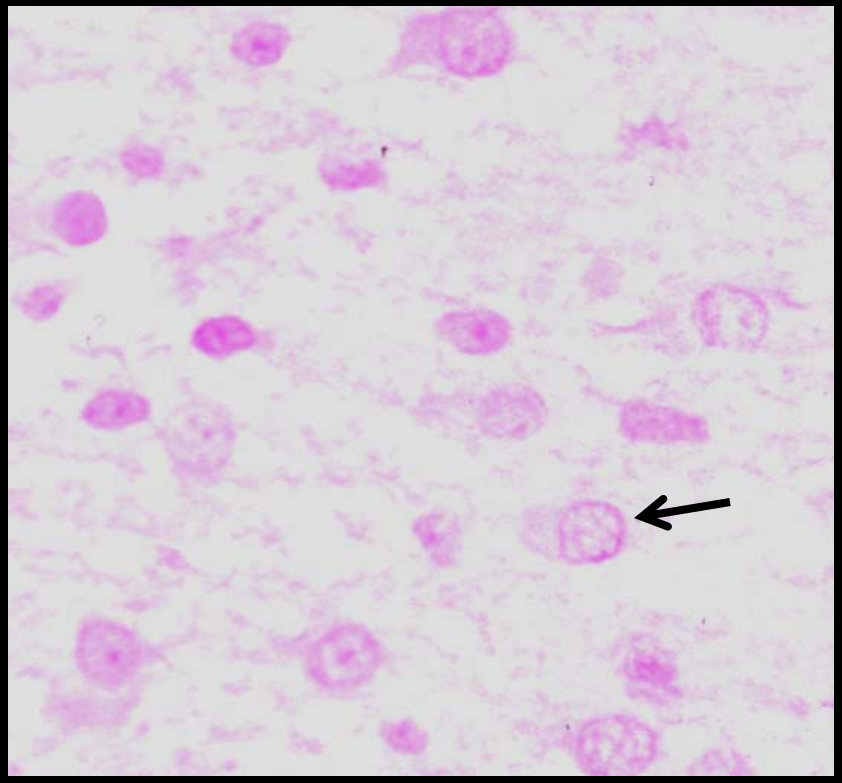
(D)



(-) staining with TUNEL in neonatal rabbit brain after following 2 hours of global hypoxia



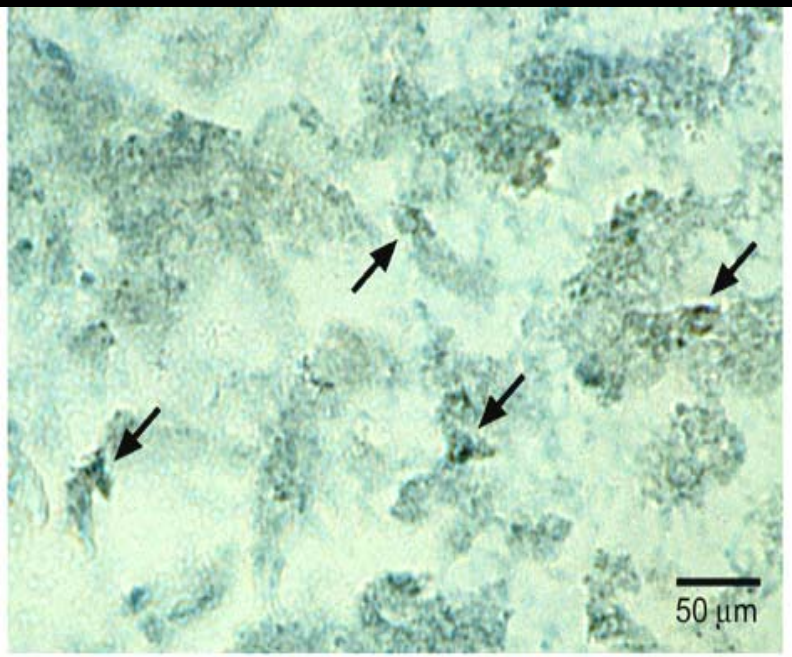
Neurons



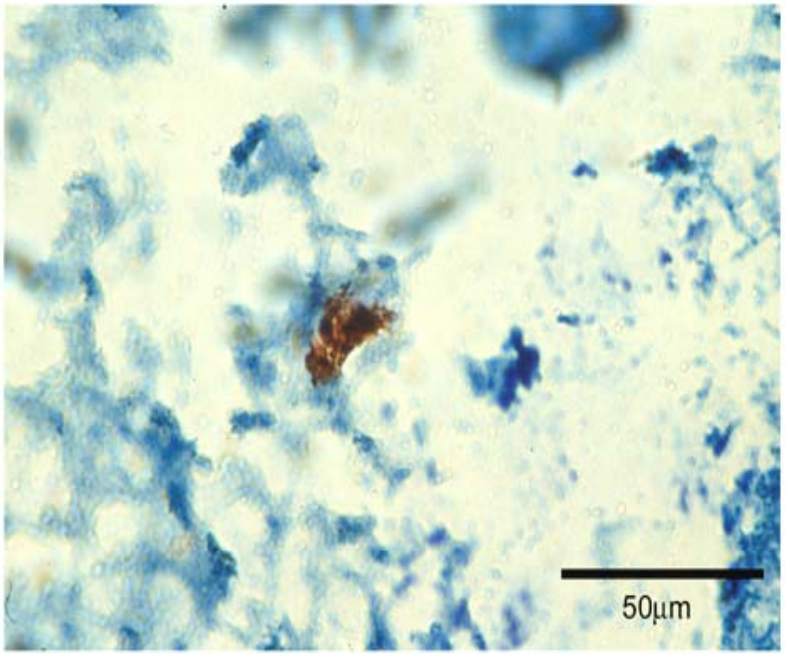
Astrocytes

Neonatal Rabbit Cerebral Hypoxia

A) Co-localization of annexin and neuronal marker (MAP2B)



A

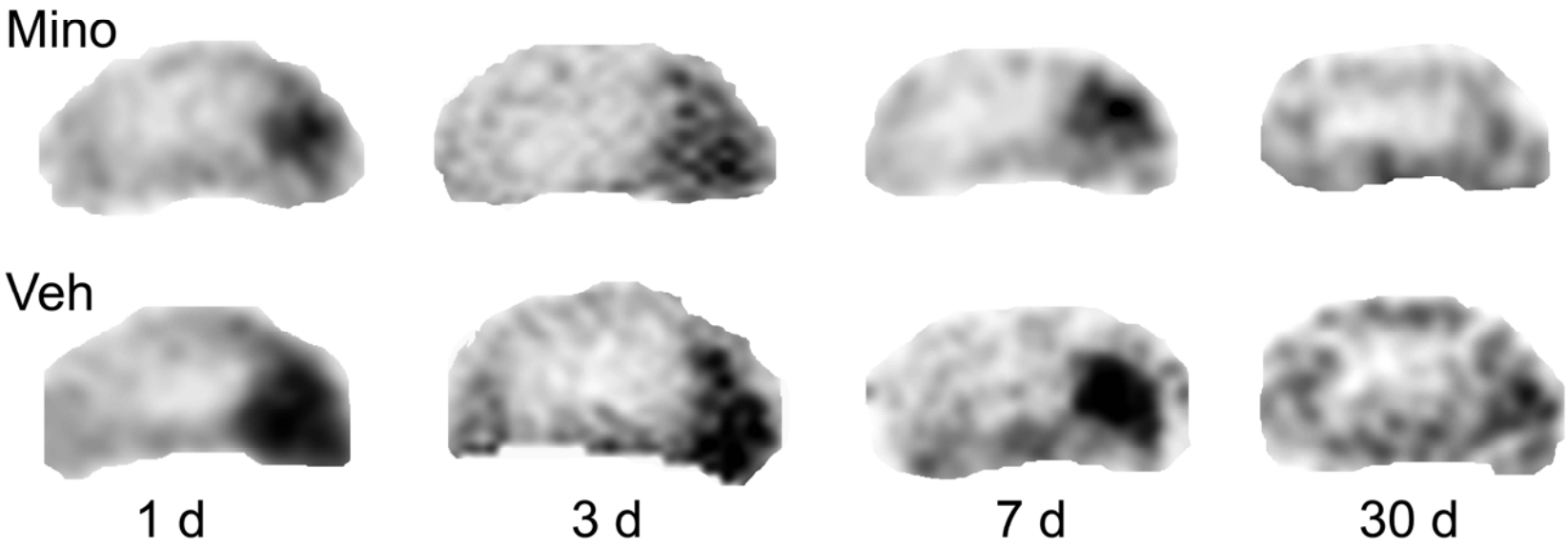


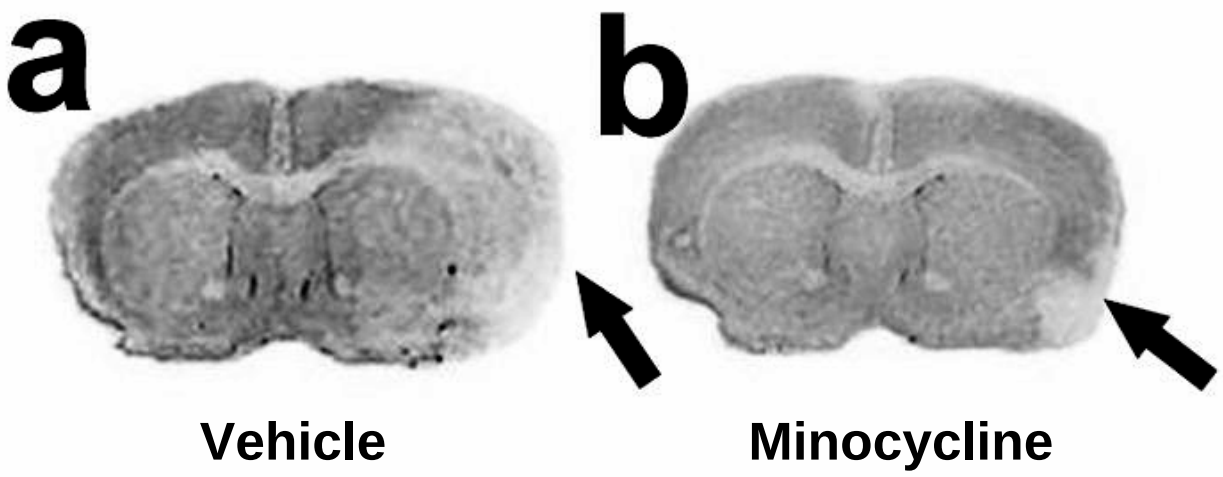
B

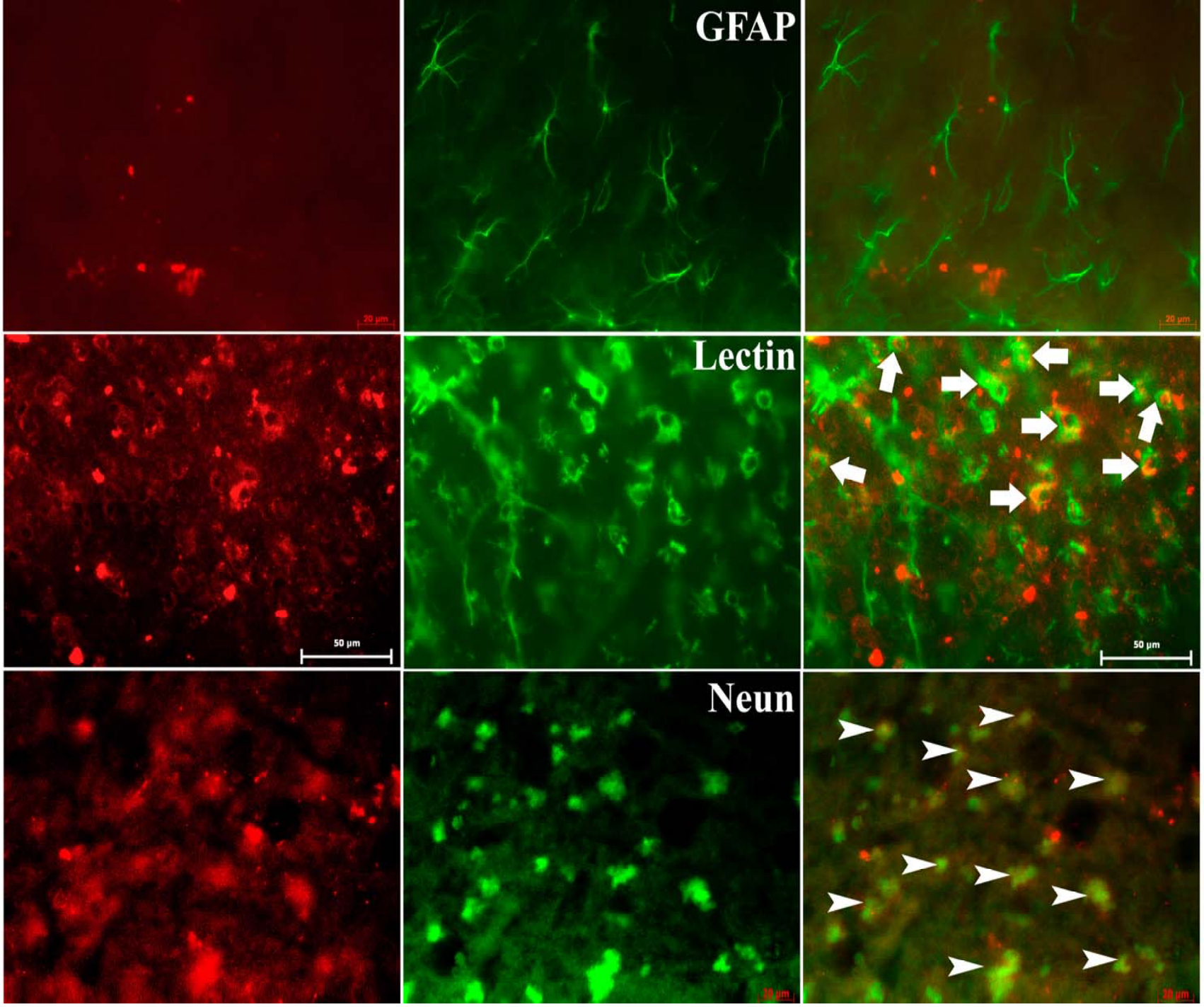
B) Localization of annexin V and astrocytic cell marker (GFAP)

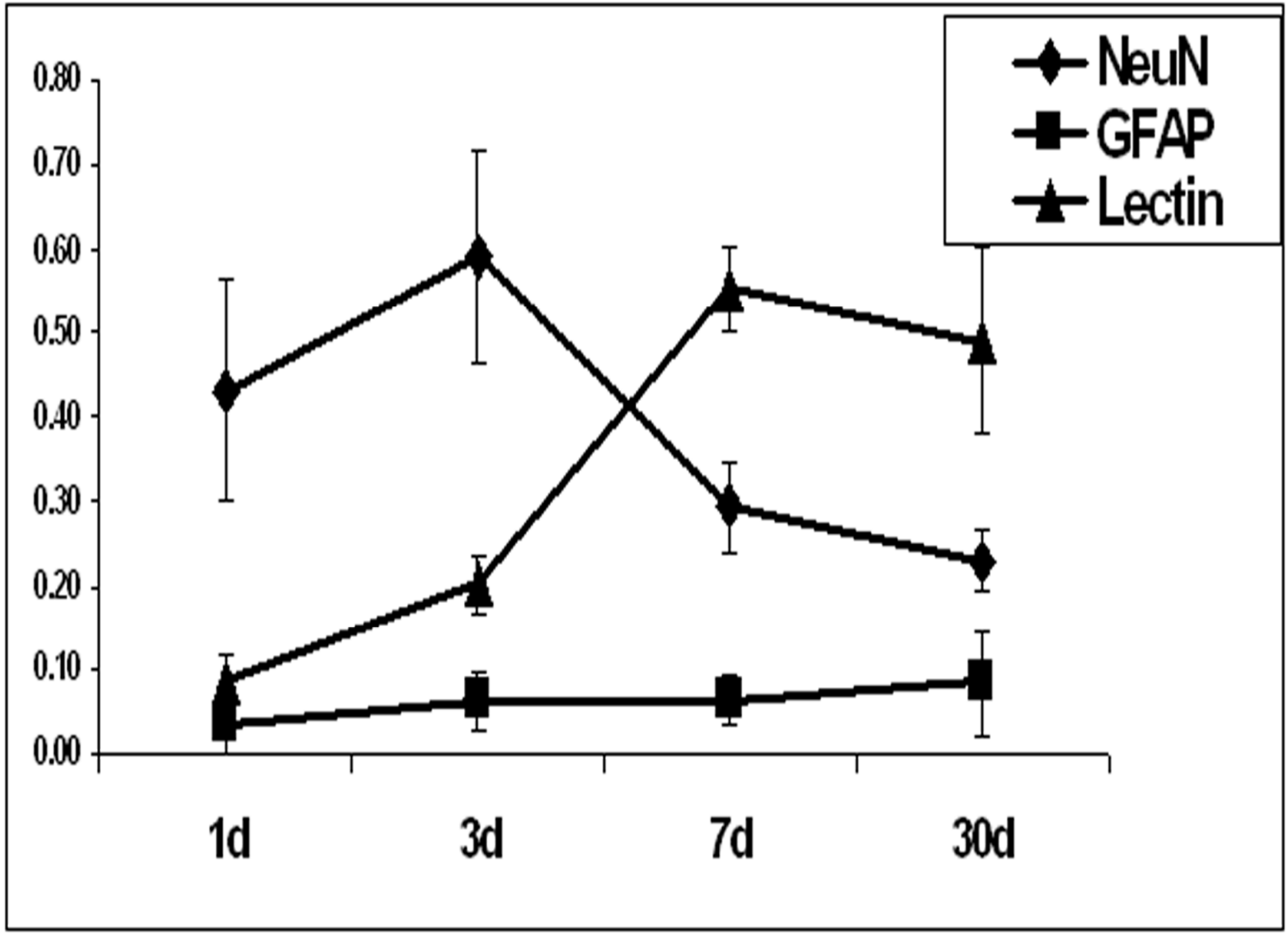
Another Example of Non-invasive Monitoring of Neuroprotective Therapy

- Minocycline is a member of the tetracycline family of antibiotics with both anti-inflammatory and anti-apoptotic bacterial properties.
- It has also been reported that minocycline has anti-apoptotic properties, via inhibition of caspase-3 [Wang CX, et al. Brain Res. 2003;963:327-9.], which and can protect against various cerebral insults [Yrjanheikki J, et al. PNAS. 1999;96(23):13496-500. & Koistinaho M, et al. Cereb Blood Flow Metab. 2005;25:460-7.].

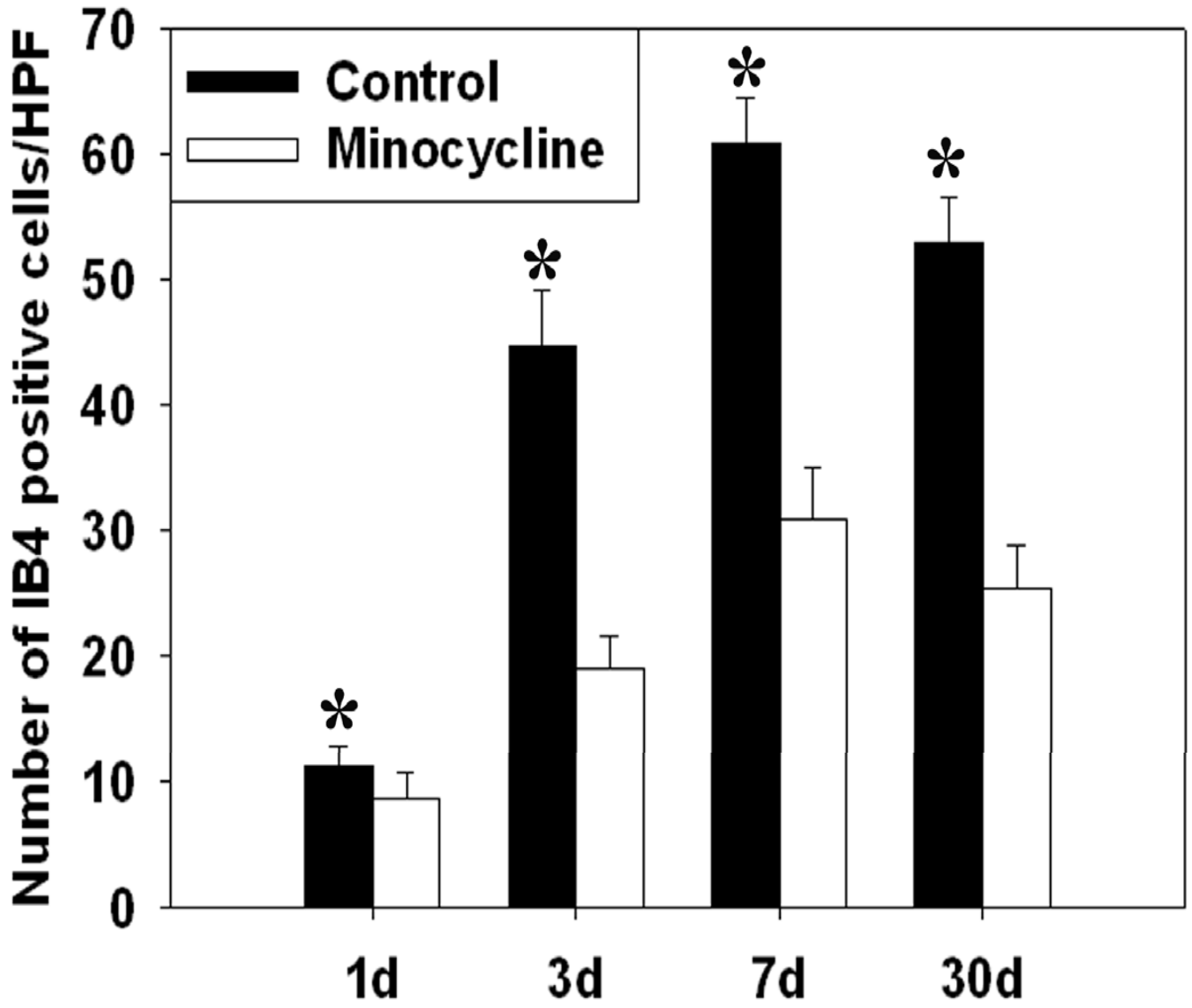

Mino
Veh
1 d
3 d
7 d
30 d


a
b
Vehicle
Minocycline

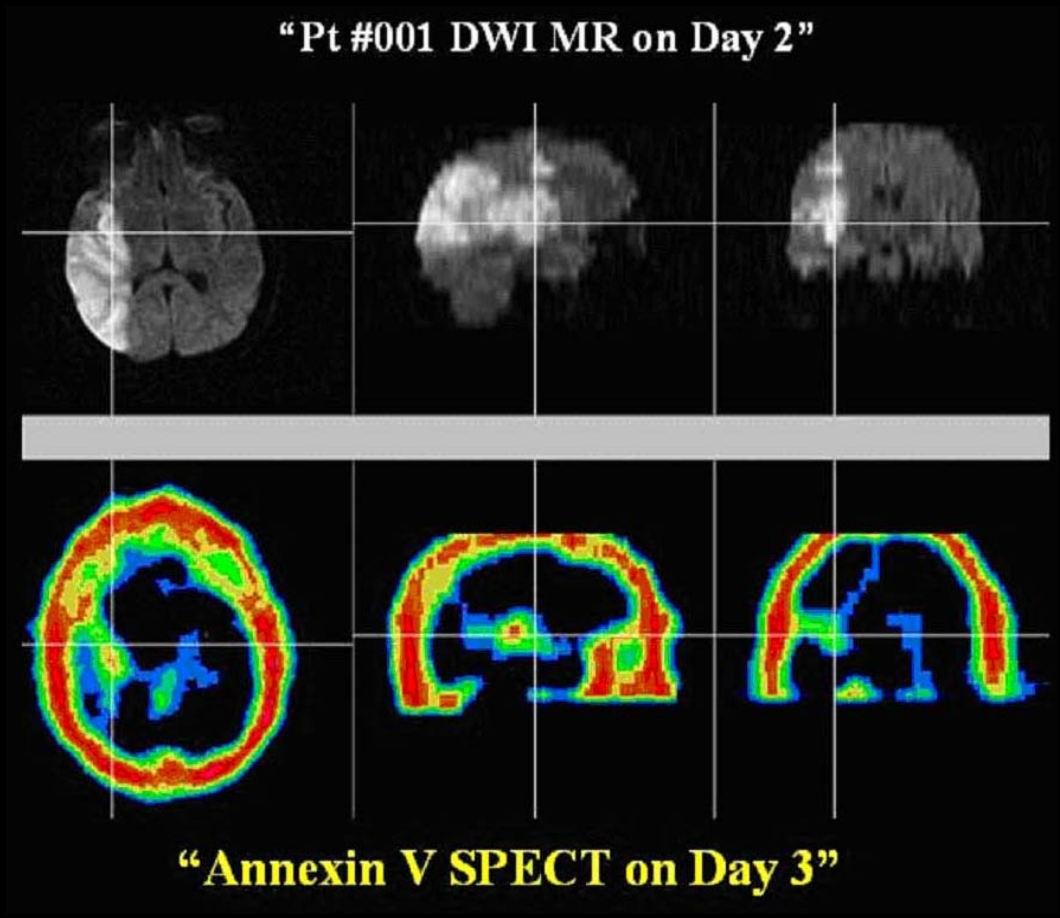

GFAP
Lectin
Neun
50 µm
50 µm


NeuN
GFAP
Lectin
0.80
0.70
0.60
0.50
0.40
0.30
0.20
0.10
0.00
1d
3d
7d
30d

Minocycline treatment reduces microglial activation

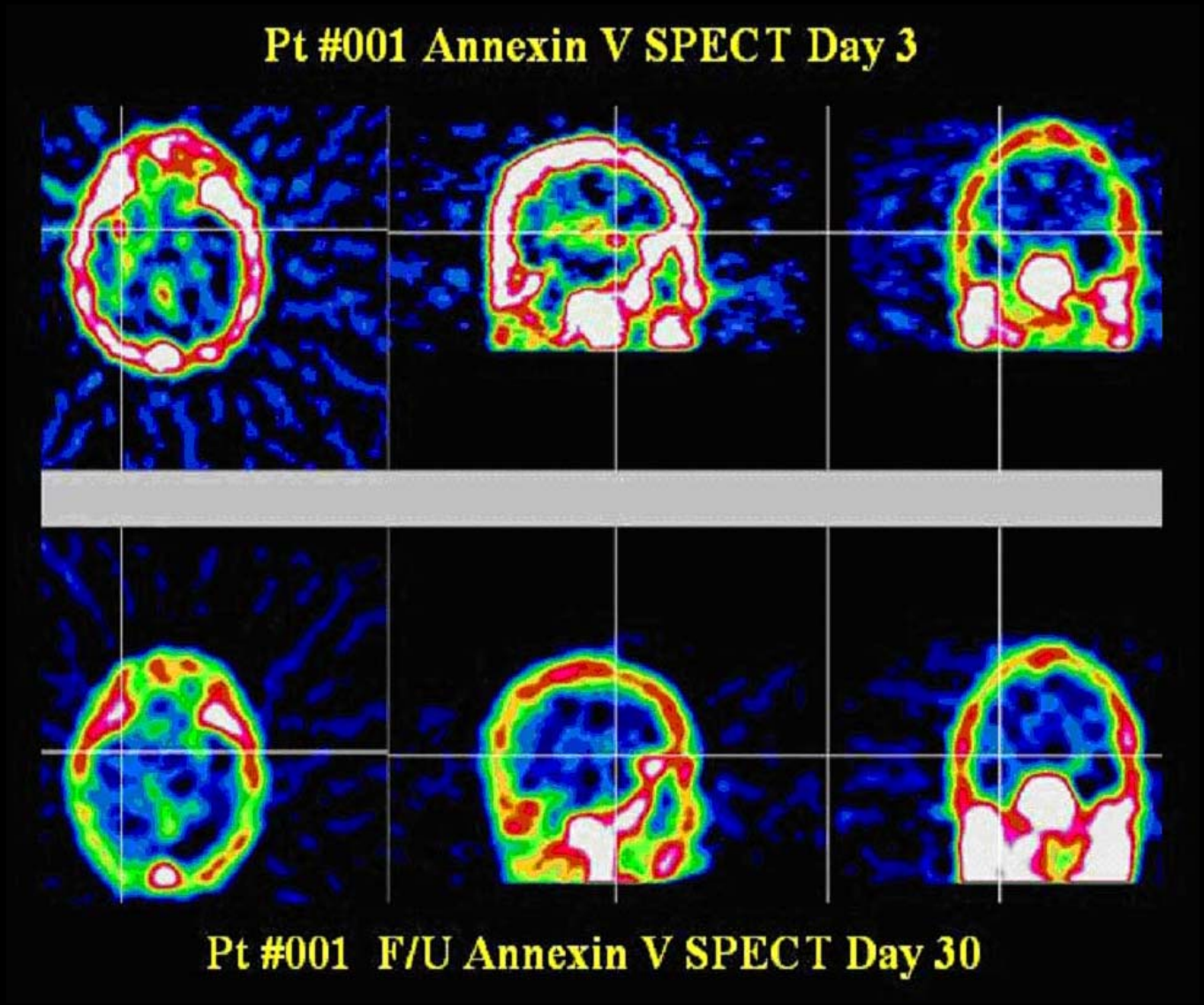


Acute Stroke

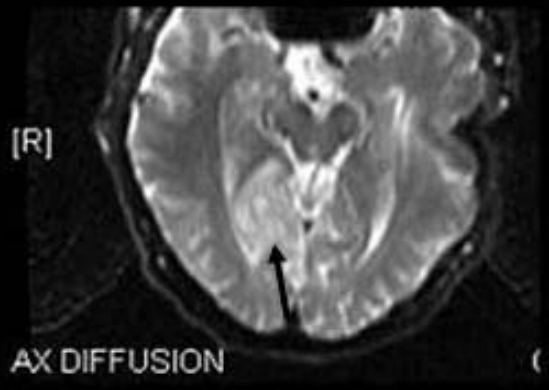


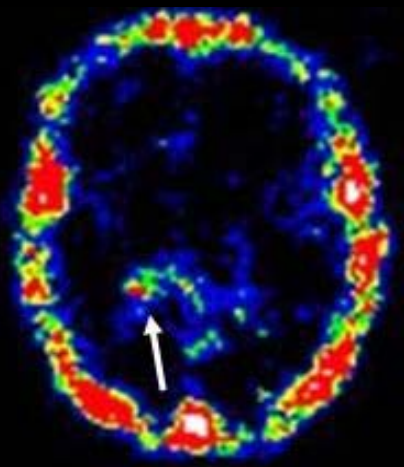
Blankenberg FG, Kalinyak J, Liu L, Koike M, Cheng D, Goris ML, Green A, Vanderheyden JL, Tong DC, Yenari M. (99m)Tc-HYNIC-annexin V SPECT imaging of acute stroke and its response to neuroprotective therapy with anti-Fas ligand antibody. *Eur J Nucl Med Mol Imaging*. 2006 Feb 14;:1-9

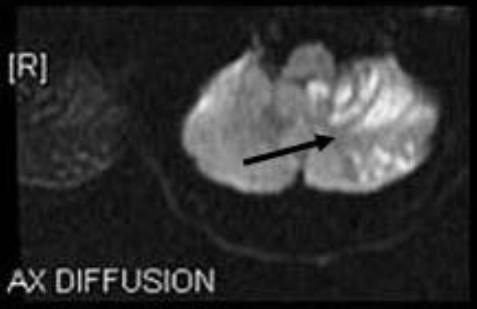
Acute vs Subacute Stroke

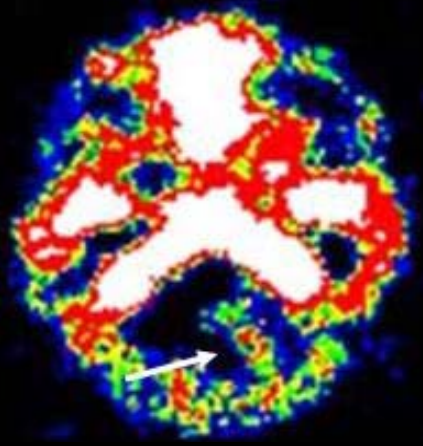


Multifocal Stroke: Age of Cerebellar Lesion Indeterminate on DWI and Acute Occipital Visual Field Loss









Alzheimer's vs Multi-infarct Dementia

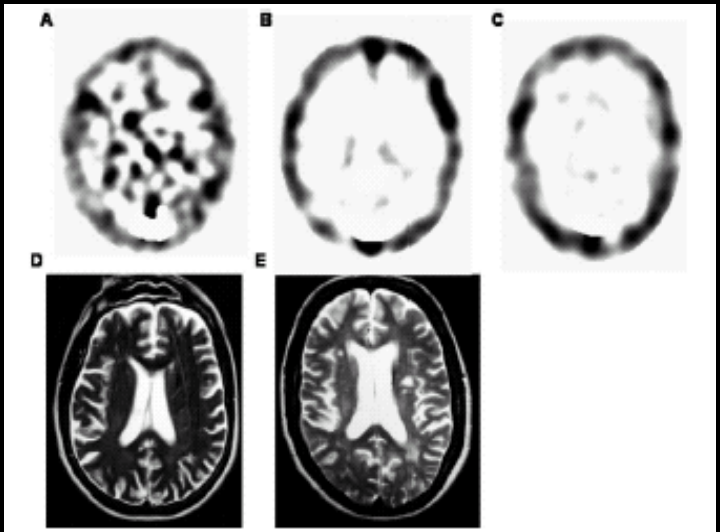
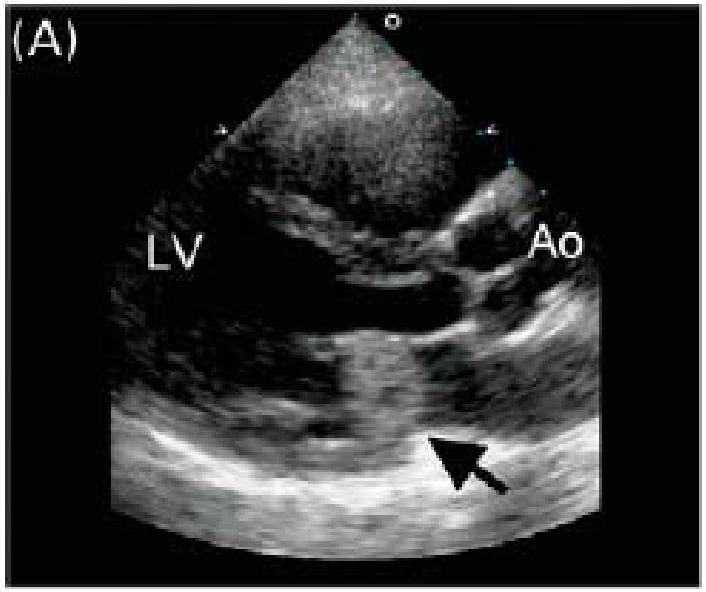


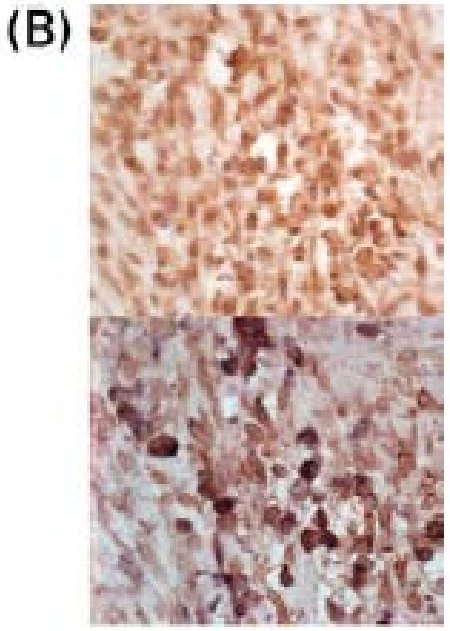
Figure. Representative transaxial SPECT slice from a patient with Alzheimer dementia (AD) (A) shows multiple foci of annexin uptake (Mini-Mental State Examination [MMSE] score = 11) and a corresponding T2-weighted magnetic resonance (MR) image (D) showing mild atrophy. Another patient (B) with non-AD dementia (multi-infarct dementia) shows minimal background activity (MMSE score = 13), and the corresponding T2-weighted MR image (E) shows abnormal signals in the periventricular white matter consistent with diffuse ischemic changes. The SPECT image of a control subject is also presented (C).

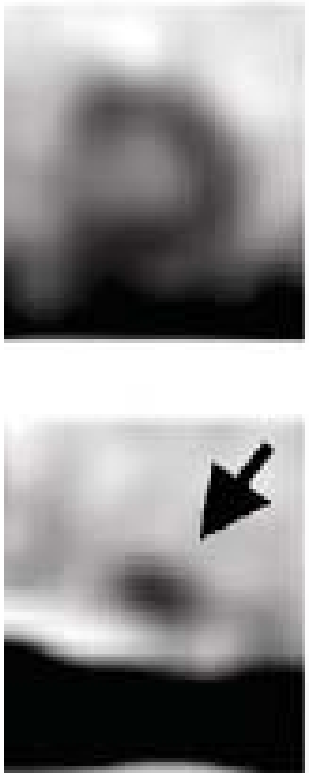
Lampl Y, Lorberboym M, **Blankenberg FG**, Sadeh M, Gilad R. Annexin V SPECT imaging of phosphatidylserine expression in patients with dementia. *Neurology*. 2006;66:1253-1254.

Annexin V Imaging on Positive on Day 1 /

Blood Cultures not positive till Day 9 for Streptococcal Endocarditis

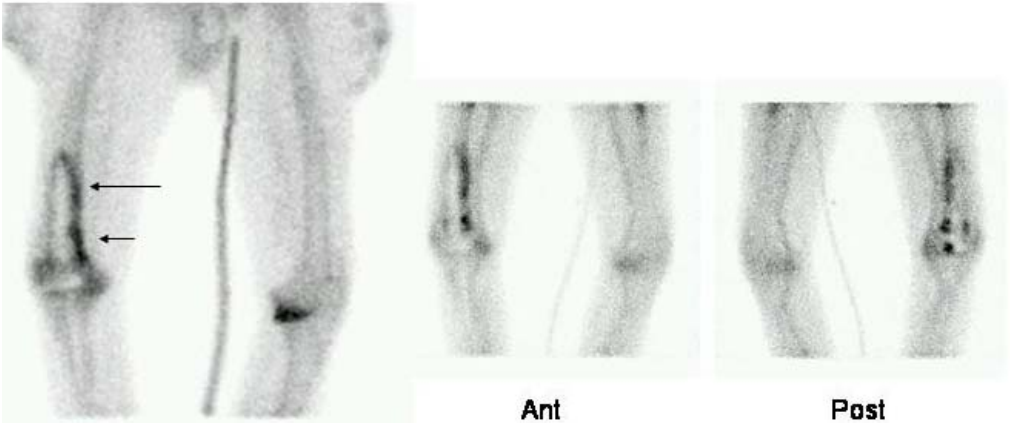






Kietselaer BLJH, et al. European Heart Journal 2007 28(8):948

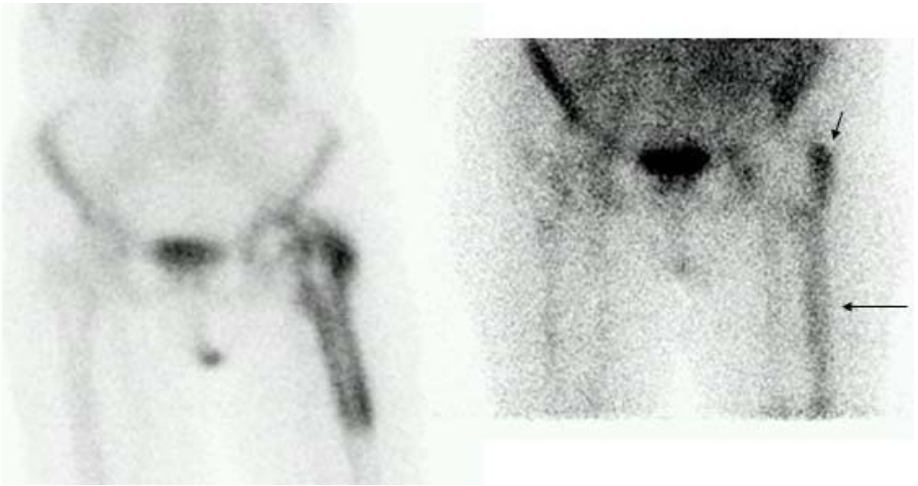
False Positive Bone Scan/ True Negative Annexin V Scan



A 76 year old man with a painful right knee prosthesis and signs of loosening on plain x ray.

A 79 year old woman with a right hip prosthesis for 15 years.

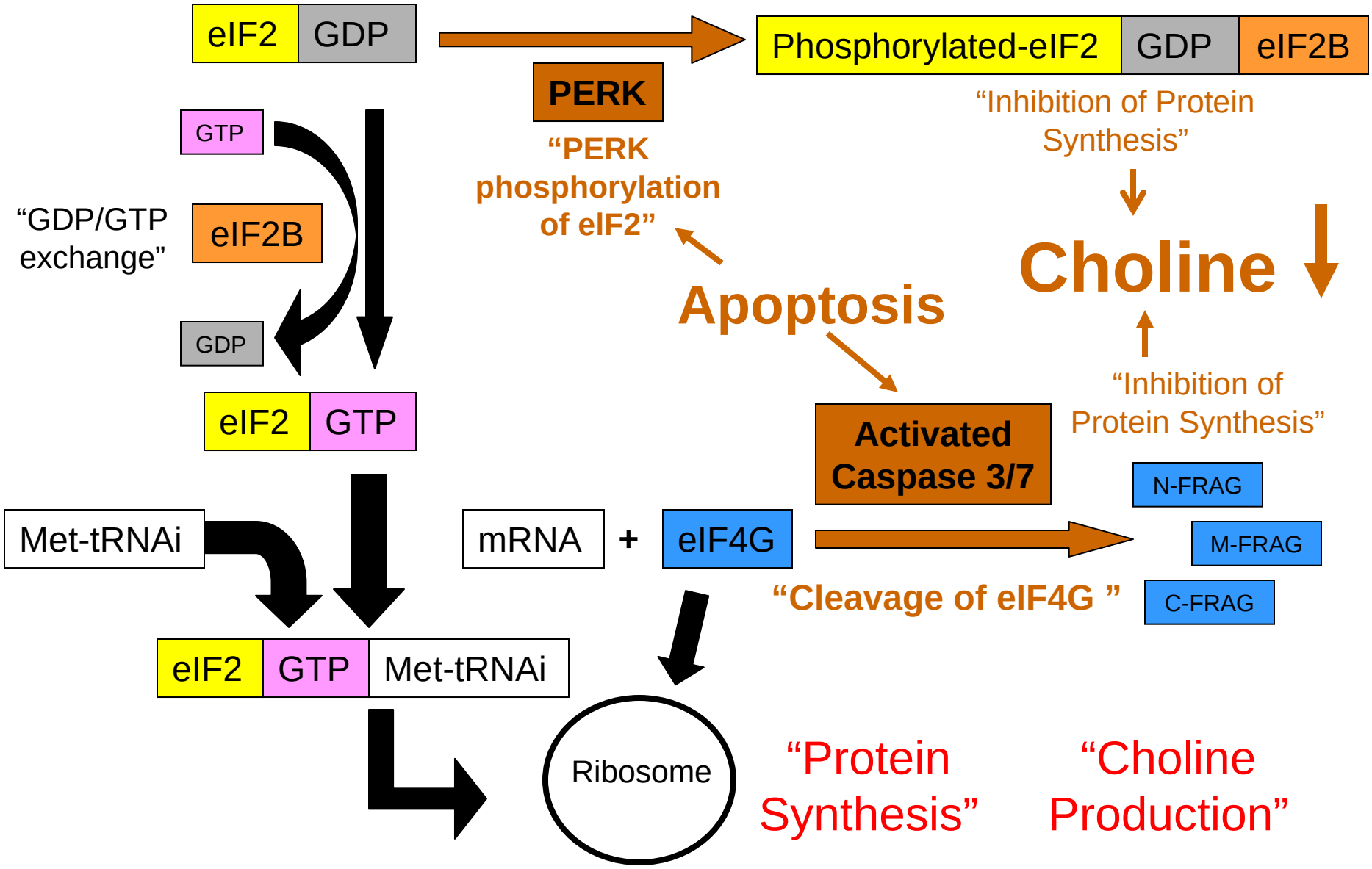
True Positive Bone Scans/ True Positive Annexin V Scans



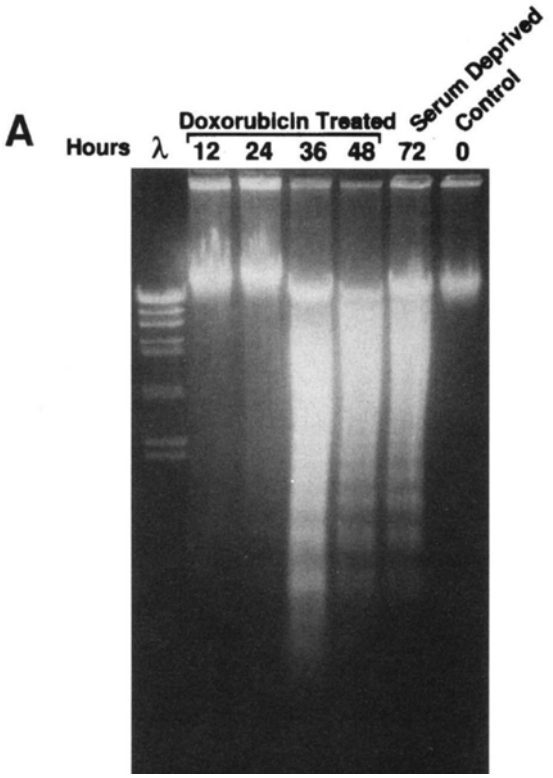
A 78 year old woman with a history of a sub-capital fracture of her left femur, leading to AVN and THR.

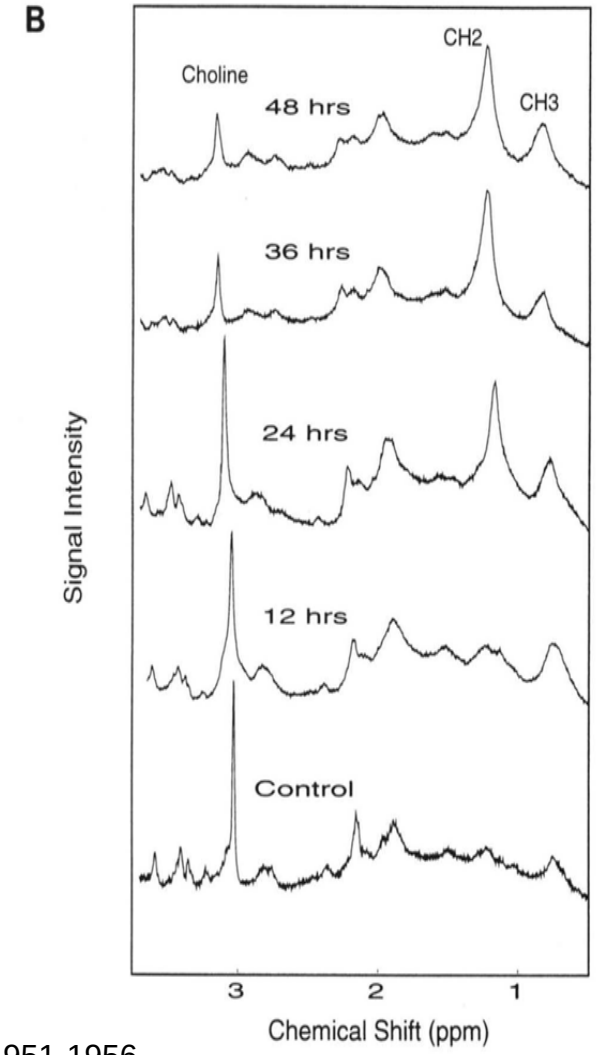
"Initiators" of Protein Synthesis **eIFs = [eukaryotic initiation factors-2, 2B and 4G]**

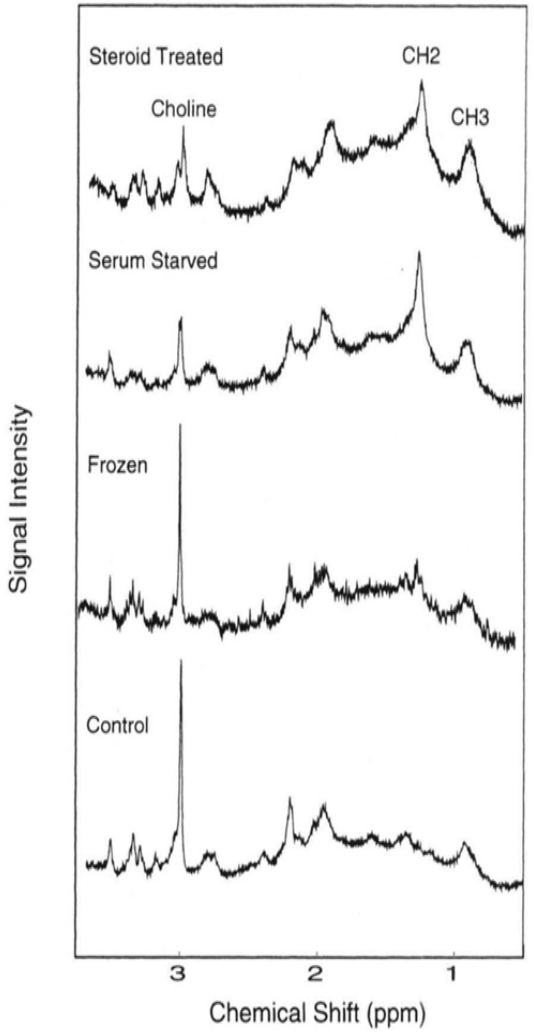
Inhibitor of Protein Synthesis **PERK = [protein kinase RNA (PRK);PRK like ER kinase]**



Detection of Apoptosis with In Vitro Lipid Proton Spectroscopy

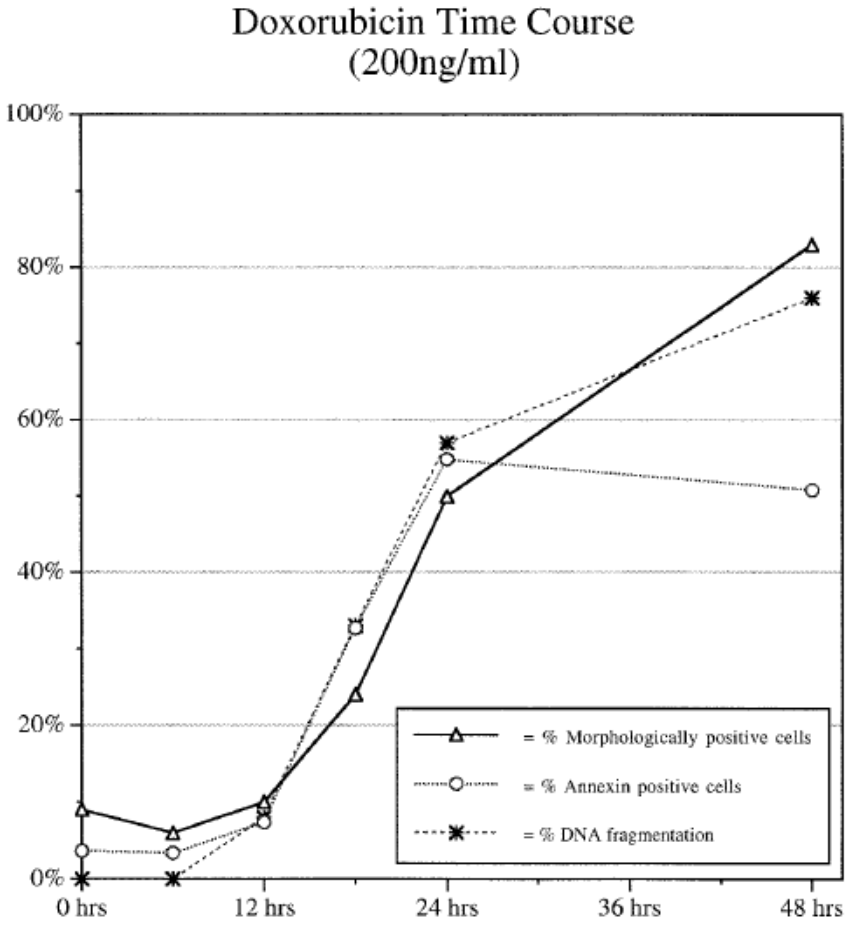




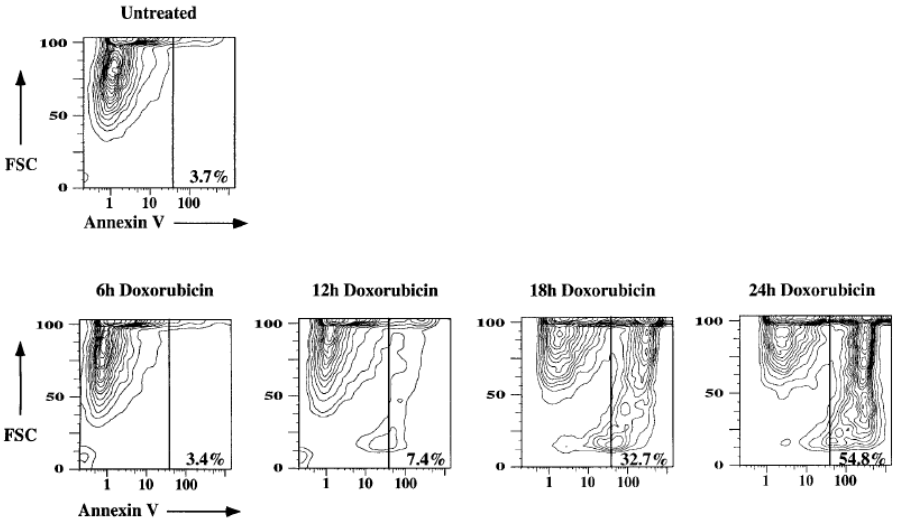


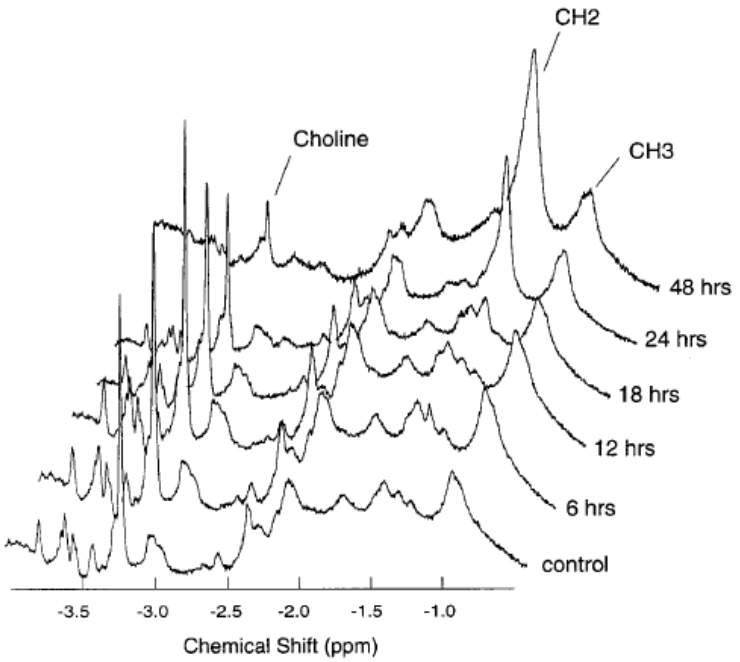
***Blood,* Vol 87, No** 5 (March 11) 1996: **pp** 1951-1956

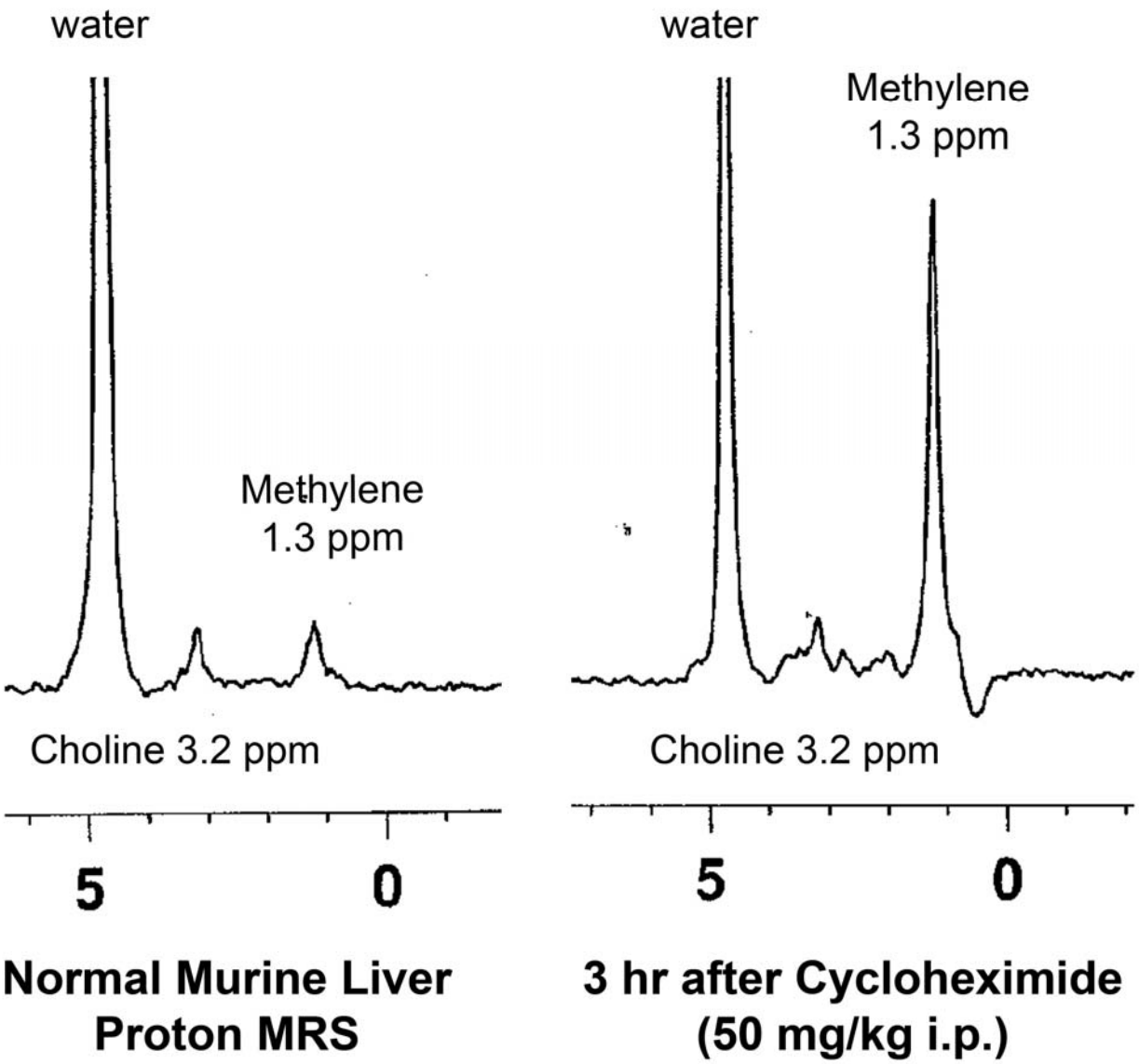
Quantification of Apoptosis with In Vitro Lipid Proton Spectroscopy



Blood, Vol 89, No 10 (May 15), 1997: pp 3778-3786

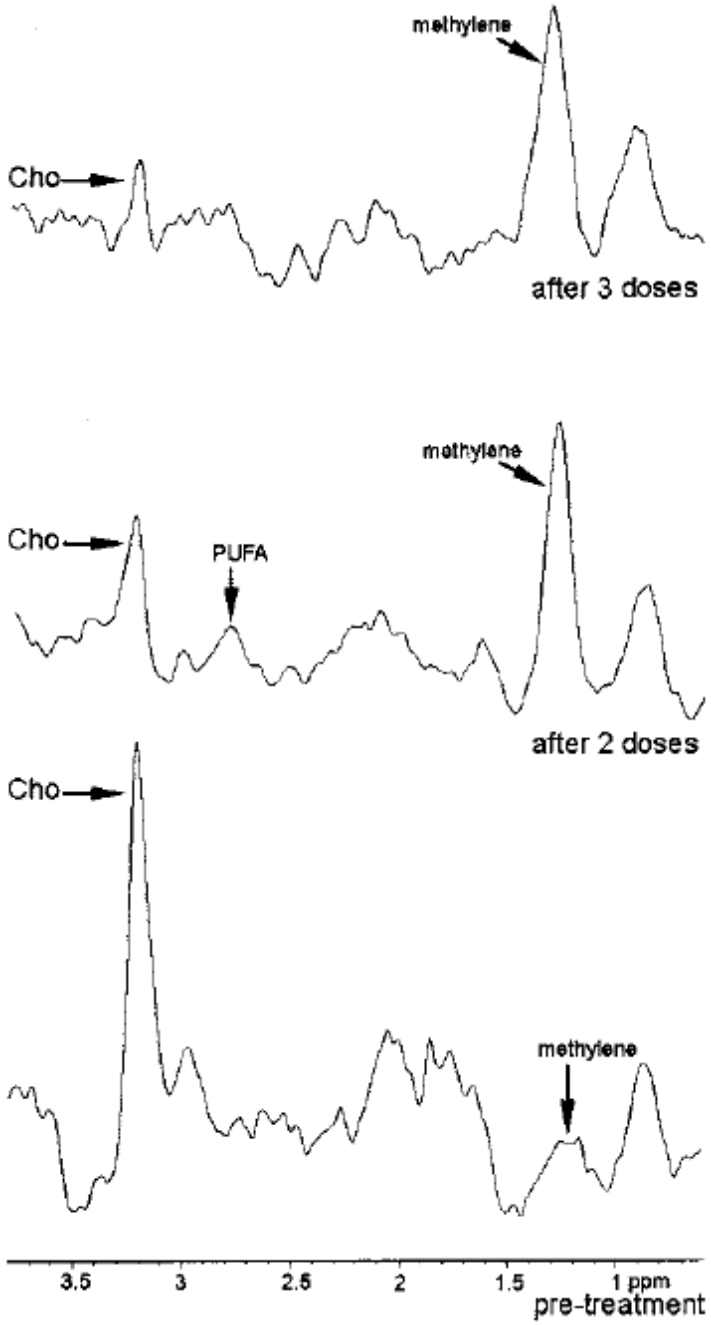


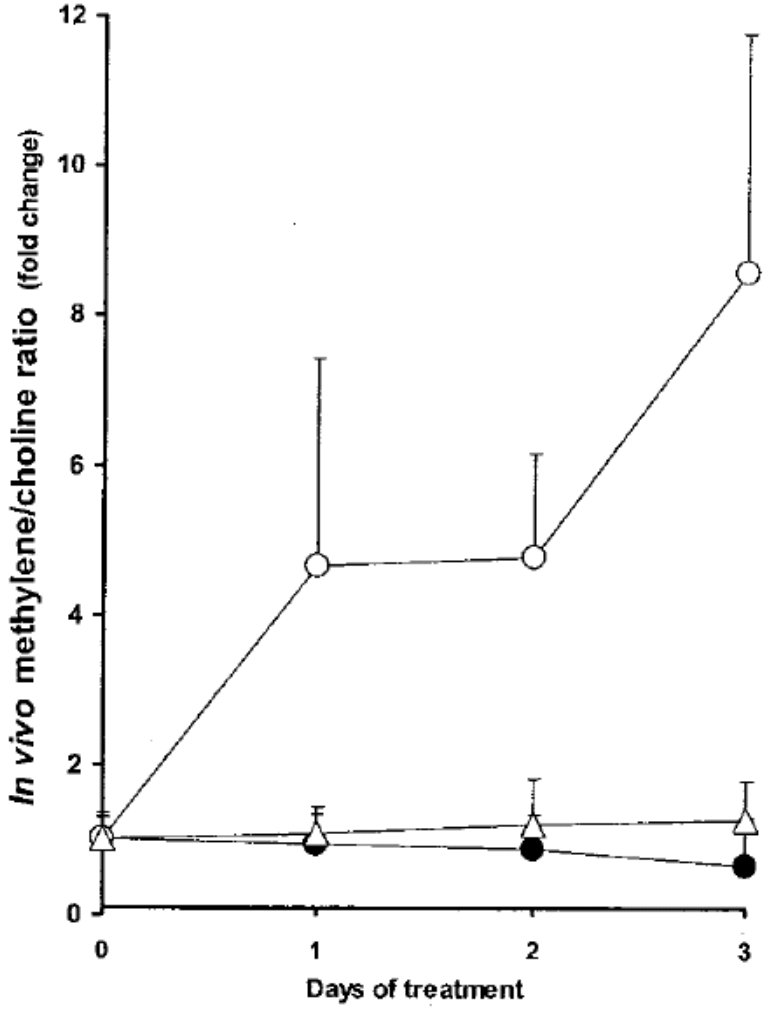




Courtesy of Helen D'Arceuil, Brain Ross, Pratip Bhattacharya / April 2010

HMRI Spectroscopy Unit : Calfornia Institute of Technology / Pasedena, CA



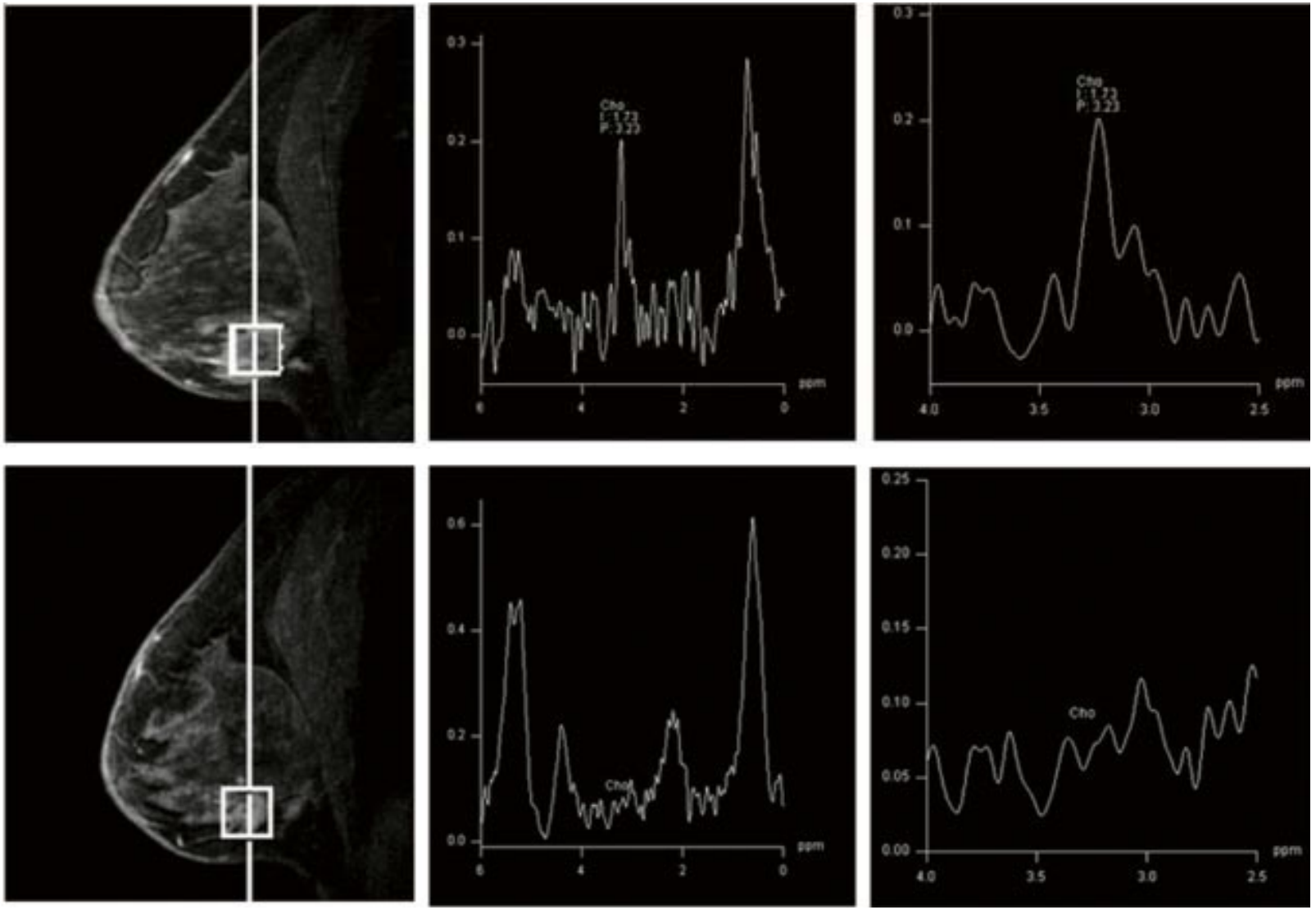


From: ***Lindskog M. et al. 1H-MRS in experimental neuroblastoma models in vivo. JNCI 2004;96:1457–66.***

Sagittal contrast-enhanced fat suppressed MR images & MR spectra of the right breast in a 32-year-old woman with invasive ductal carcinoma.

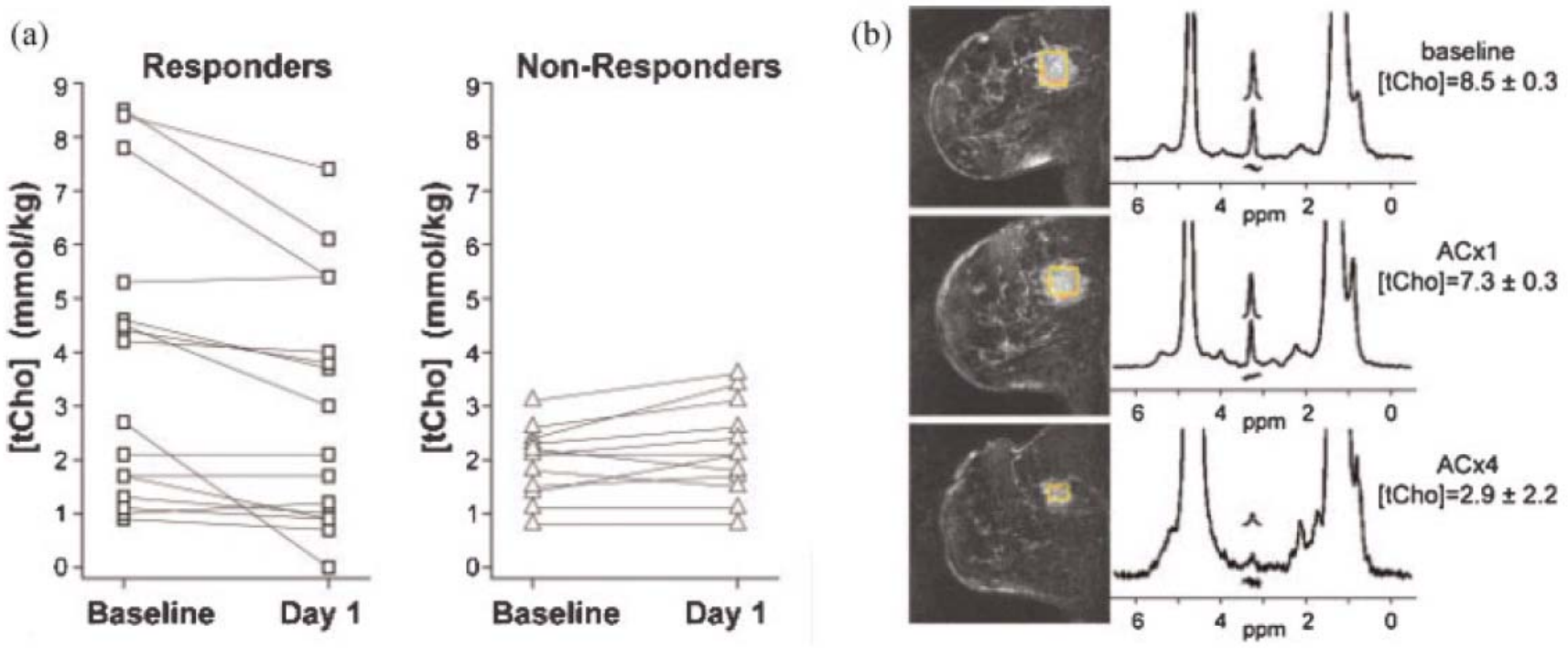
Pre Rx Baseline

After 2 cycles of Therapy



From: Tozaki M, et al. J. Magn. Reson. Imaging 2008;28:420–427.

Monitoring response to treatment quantitative breast MRS at 4 T.



75% of the objective responders showed a decrease in [tCho] at day 1 after therapy

whereas 92% of non-responders showed no change or an increase at the same time point.

From: Haddadin IS et al. NMR Biomed. 2009; 22: 65–76

Conclusions from the Most Recent Proton MRS Studies

Apoptosis correlates with a rapid (within 3-48 hours) "increase" in CH_2/CH_3 peak intensity ratio as well as an accumulation of TAGs (triacylglycerides) in cytoplasmic lipid droplets.

Apoptosis also correlates with a rapid (within 12-48 hours) "decrease" in total choline signal intensity (t-Cho at 3.2 ppm) that directly reflects a decrease in global protein synthesis within an apoptotic cell.

Conclusions from the Most Recent Proton MRS Studies

The ratio CH_2 / t-Cho has the highest dynamic range of any MRS variable taking advantage of increases in methylene (mobile lipid) and decreases in total choline that both coincide with the early stages (*within the first 3 to 48 hours of the start of therapy*) of apoptotic cell death.