PROCEED to Chronic Pancreatitis

(Prospective Evaluation of Chronic Pancreatitis for Epidemiologic and Translational Studies)

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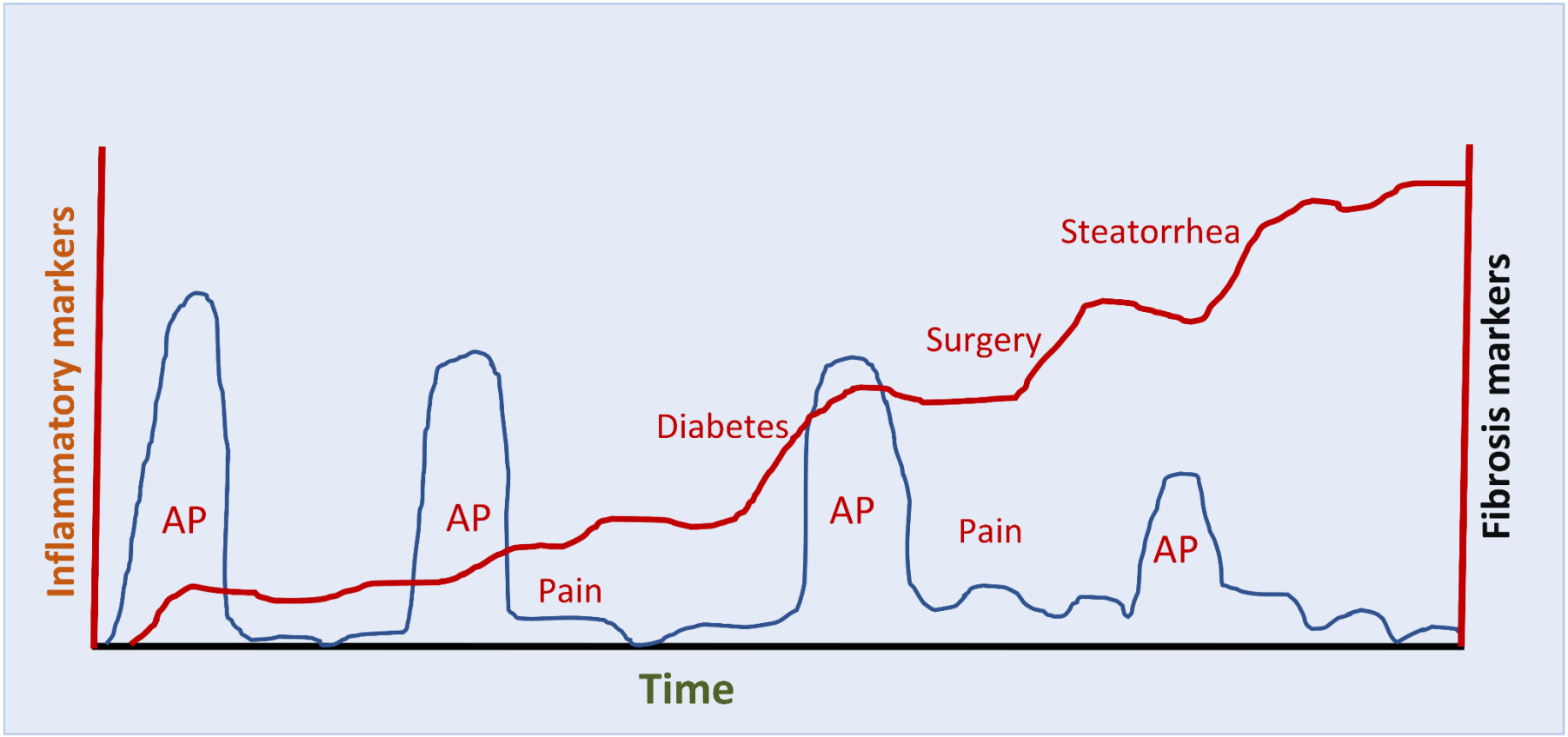
Lecture Outline

- RAP – CP Natural History
- Research Opportunities and Gaps: Discovery
- Future Directions: Validation, Clinical Implementation

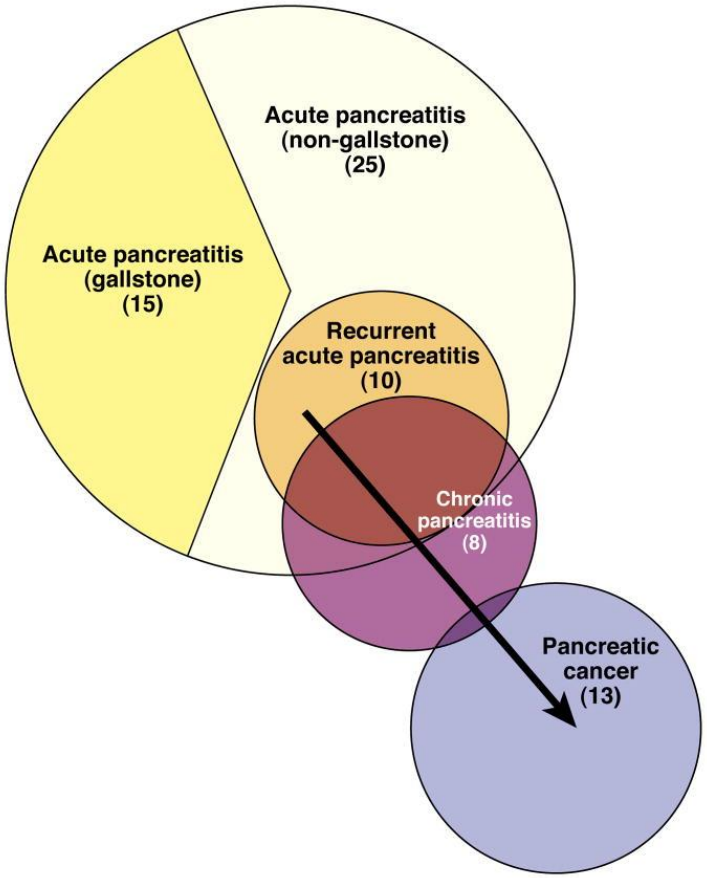
Natural history of RAP - CP results in progressive fibrosis and loss of function



Modified from: Colombel J et al. Gastroenterology 2017;152:351-61

Recurrent Acute Pancreatitis

- ≥2 episodes of AP with resolution of symptomatic and imaging abnormalities between episodes
- Occurs in ~20% of AP patients
- **RAP is the strongest risk factor for progression to CP**
  - HR of 4.57 (95% CI 3.40-6.14)



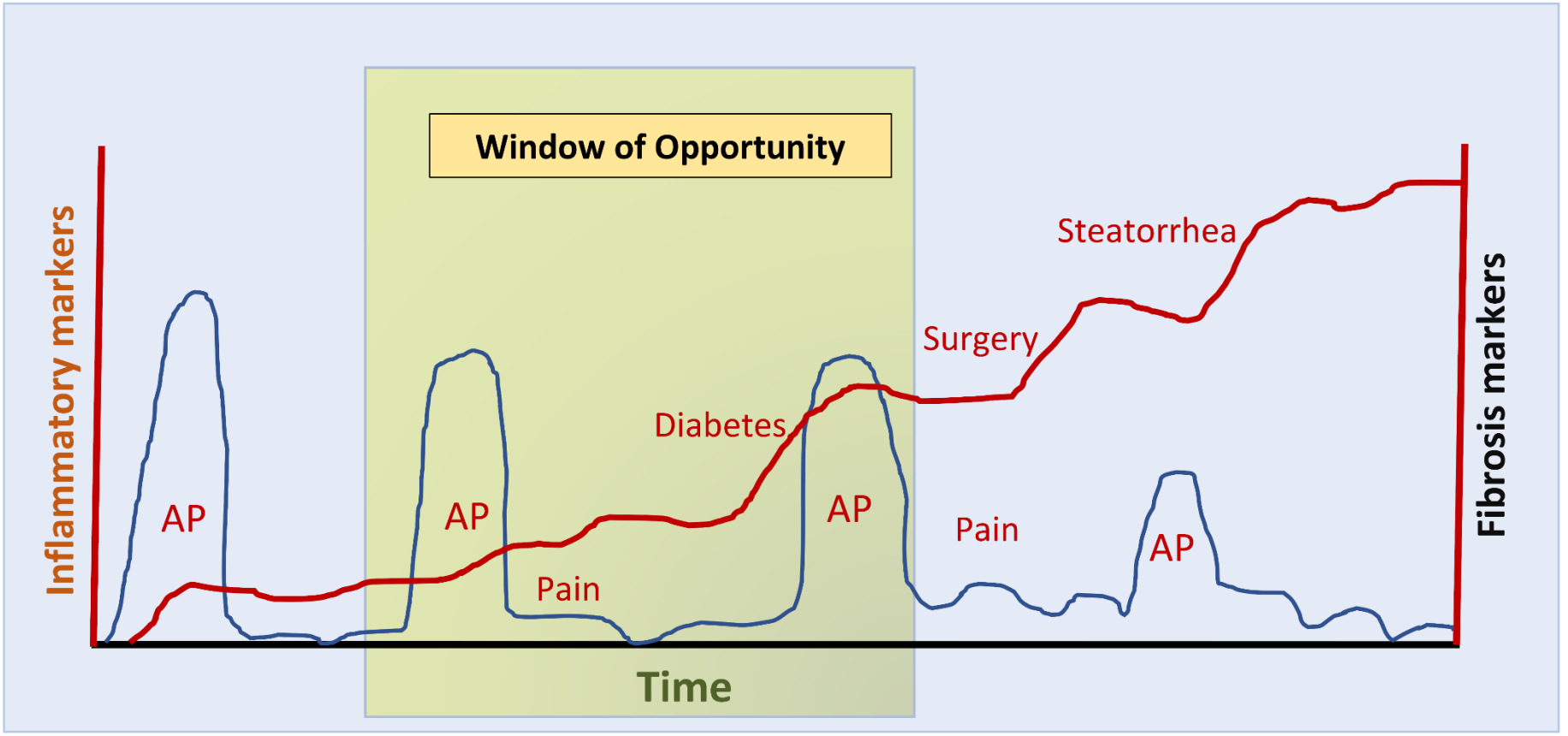
Yadav. Gastroenterology 2013;144:1252.
Yadav. Am J Gastroenterol 2012; 107:1096.
Lankisch. Am J Gastroenterol 2009;104:2797.

Progression from AP to CP: Population studies

Country	Years	Sample size	Follow up (years)	Etiology	CP (%)
Netherlands	1985-90	9579	5	All	**6**
Germany	1997-2004	532	7.8 (median)	All	4
				Alcohol	13
				Idiopathic	None
Japan	1987	714*	13 (min.)	All	**15**
				Alcohol	26
				Idiopathic	13
Denmark	1977-82	352	Until 2008	All	**24**
				Alcohol	32
				Non-alcohol	20
USA	1996-2005	6010	~4	All	**6**
				Alcohol	12
				Non-alcohol	4

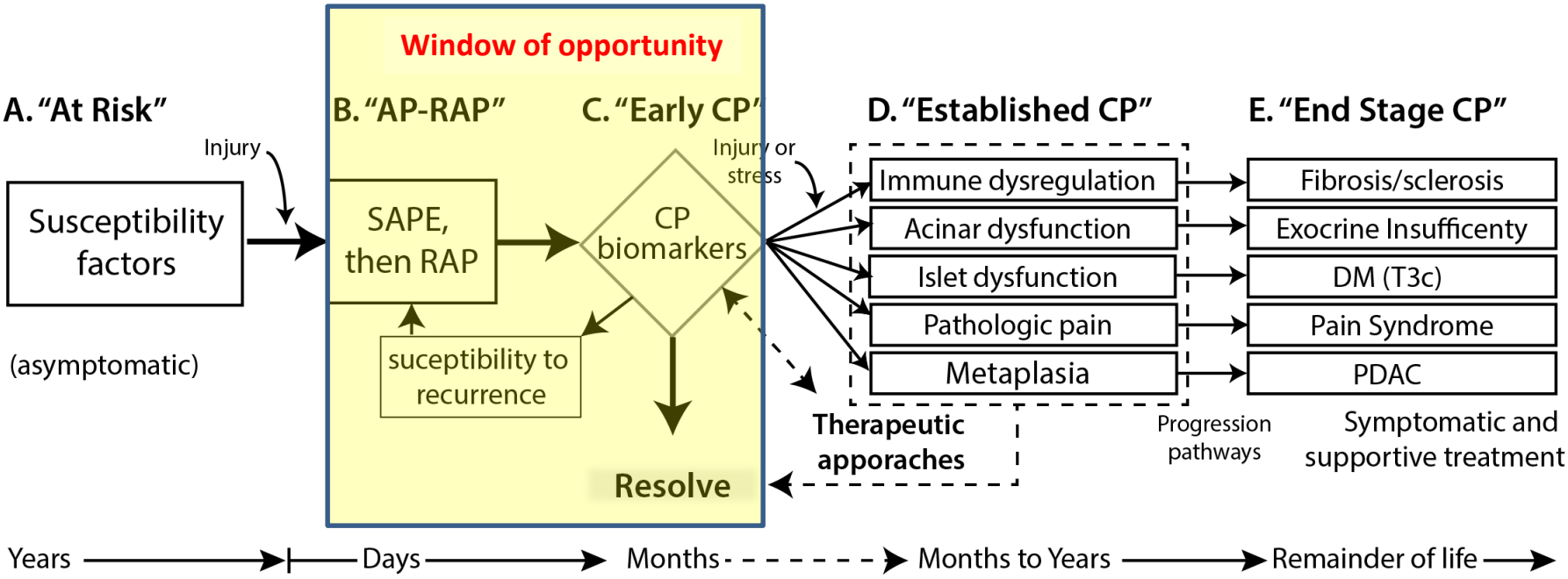
*Total sample – 2533 Scand J Gastro 2000; Am J Gastro 2009; Clin Gastro Hepatol 2009; Pancreas 2011; Am J Gastro 2011

Conceptual framework – CP



Modified from: Colombel J et al. Gastroenterology 2017;152:351-61

Mechanistic Definition



- **Biomarker Discover / Development**
- **Yellow Zone – RAP, "Early CP"**

Whitcomb, DC, et al., Pancreatology 2016

Chronic Pancreatitis in the 21st Century - Research Challenges and Opportunities

Summary of a National Institute of Diabetes and Digestive and Kidney Diseases Workshop

Aliye Uc, MD, Dana K. Andersen, MD,† Melena D. Bellin, MD,‡ Jason I. Bruce, PhD,§ Asbjørn M. Drewes, MD, PhD, DMSc,|| John F. Engelhardt, PhD,¶ Christopher E. Forsmark, MD,# Markus M. Lerch, MD,** Mark E. Lowe, MD, PhD,†† Brent A. Neuschwander-Tetri, MD,‡‡ Stephen J. O'Keefe, MD, MSc,§§ Tonya M. Palermo, PhD,|||| Pankaj Pasricha, MD,¶¶ Ashok K. Saluja, PhD,## Vikesh K. Singh, MD, MSc,¶¶ Eva M. Szigethy, MD, PhD,§§ David C. Whitcomb, MD, PhD,§§ Dhiraj Yadav, MD, MPH,§§ and Darwin L. Conwell, MD, MS****

Research Gaps and Opportunities

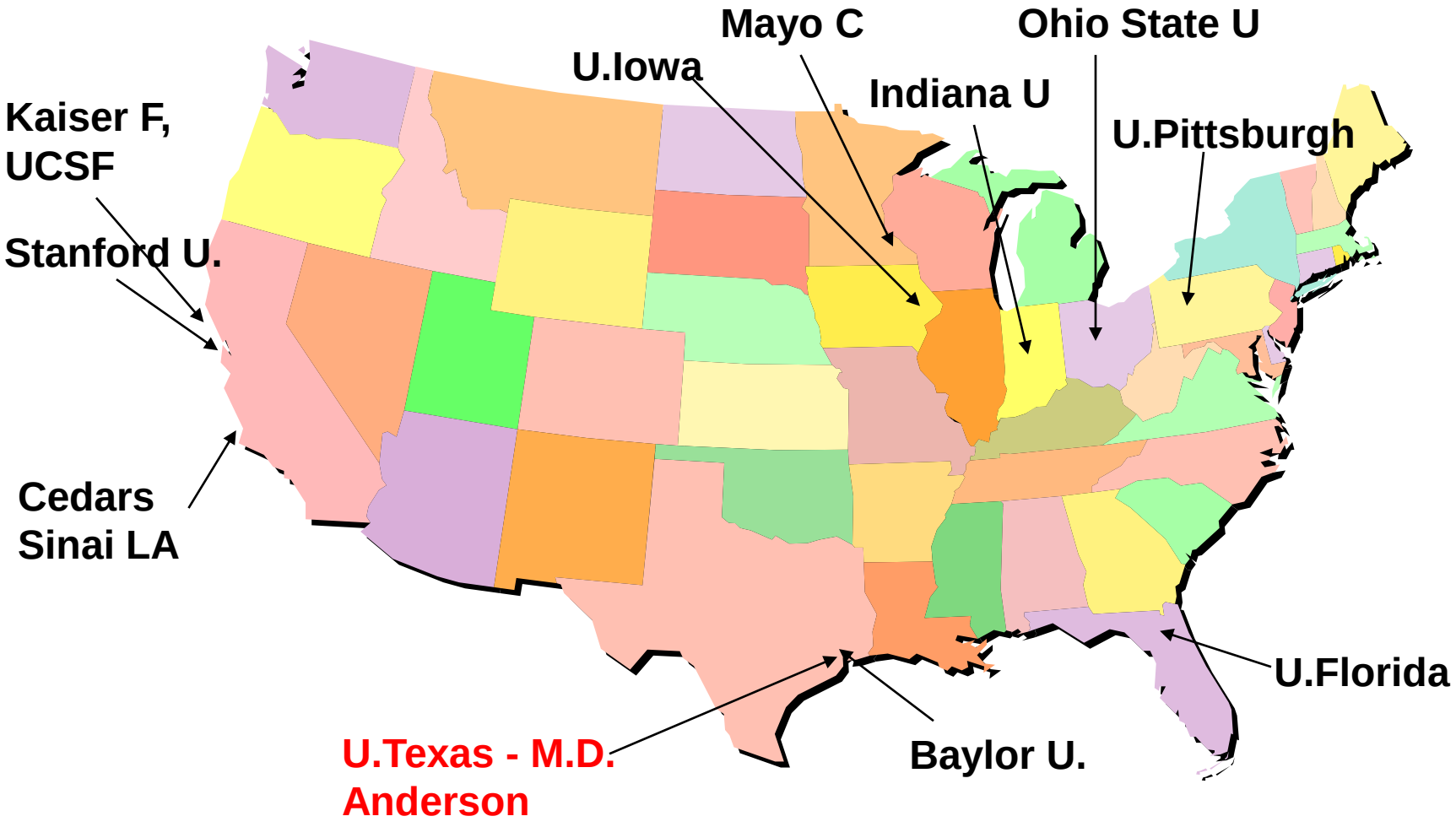
- Improve and accurate assessment of maldigestion and EPI.
- Establish simpler, less invasive tools to measure acinar and ductal cell function from more easily obtained biological specimens such as urine or blood to screen for pancreatic disease.
- Develop RAP and CP biomarkers that can be used to better de- fine the stage, determine prognosis, assess severity, and stratify patients for medical or surgical intervention using the mechanistic definition framework.
- Provide evidence-based recommendations for proper dietary intake and the requirements for PERT (initiation, dose, timing, follow-up).
- Develop enzyme products requiring fewer pills and with better compliance and potency.

Research Gaps and Opportunities

- Develop long-term primary acinar and ductal epithelial cell culture models.
- Explore co-culture models (eg, acinar-duct, duct-islet, acinar- islet) to identify factors that regulate exocrine cell function and restitution.
- Define mechanisms by which gene mutations/variants cause pancreatic inflammation, ductal cell malfunction, and acinar cell loss.
- Design novel therapies that target restoring pancreatic acinar cells and/or manipulate ductal cells (ie, gene and cell-based therapies, CRISPR/Cas9, *CFTR* correctors and potentiators).
- Develop experiments to determine the critical age and time for intervention to reestablish appropriate stem cell niches for cell-based therapies in diseases that damage the exocrine pancreas.

Uc A., Pancreas 2016.

RFA-DK- 14-027/28: Consortium for the Study of Chronic Pancreatitis Diabetes and Pancreatic Cancer



*PRO*spective Evaluation of Chronic Pancreatitis for *Epid*Emiologic and Translational Stu*D*ies

Rationale and Study Design for PROCEED From the Consortium for the Study of Chronic Pancreatitis, Diabetes, and Pancreatic Cancer

Dhiraj Yadav, MD, MPH, Walter G. Park, MD,† Evan L. Fogel, MD, MSc,‡ Liang Li, PhD,§ Suresh T. Chari, MD,|| Ziding Feng, PhD,¶ William E. Fisher, MD,# Christopher E. Forsmark, MD,** Christie Y. Jeon, ScD,†† Aida Habtezion, MD, MSc,† Phil A. Hart, MD,‡‡ Steven J. Hughes, MD,§§ Mohamed O. Othman, MD,|||| Jo Ann S. Rinaudo, PhD,¶¶ Stephen J. Pandol, MD,## Temel Tirkes, MD,*** Jose Serrano, MD, PhD,††† Sudhir Srivastava, PhD, MPH,¶¶ Stephen K. Van Den Eeden, PhD,‡‡‡ David C. Whitcomb, MD, PhD,*§§§|||||| Mark Topazian, MD,|| and Darwin L. Conwell, MD, MSc,‡‡ on behalf of the Consortium for the Study of Chronic Pancreatitis, Diabetes, and Pancreatic Cancer (CPDPC)*

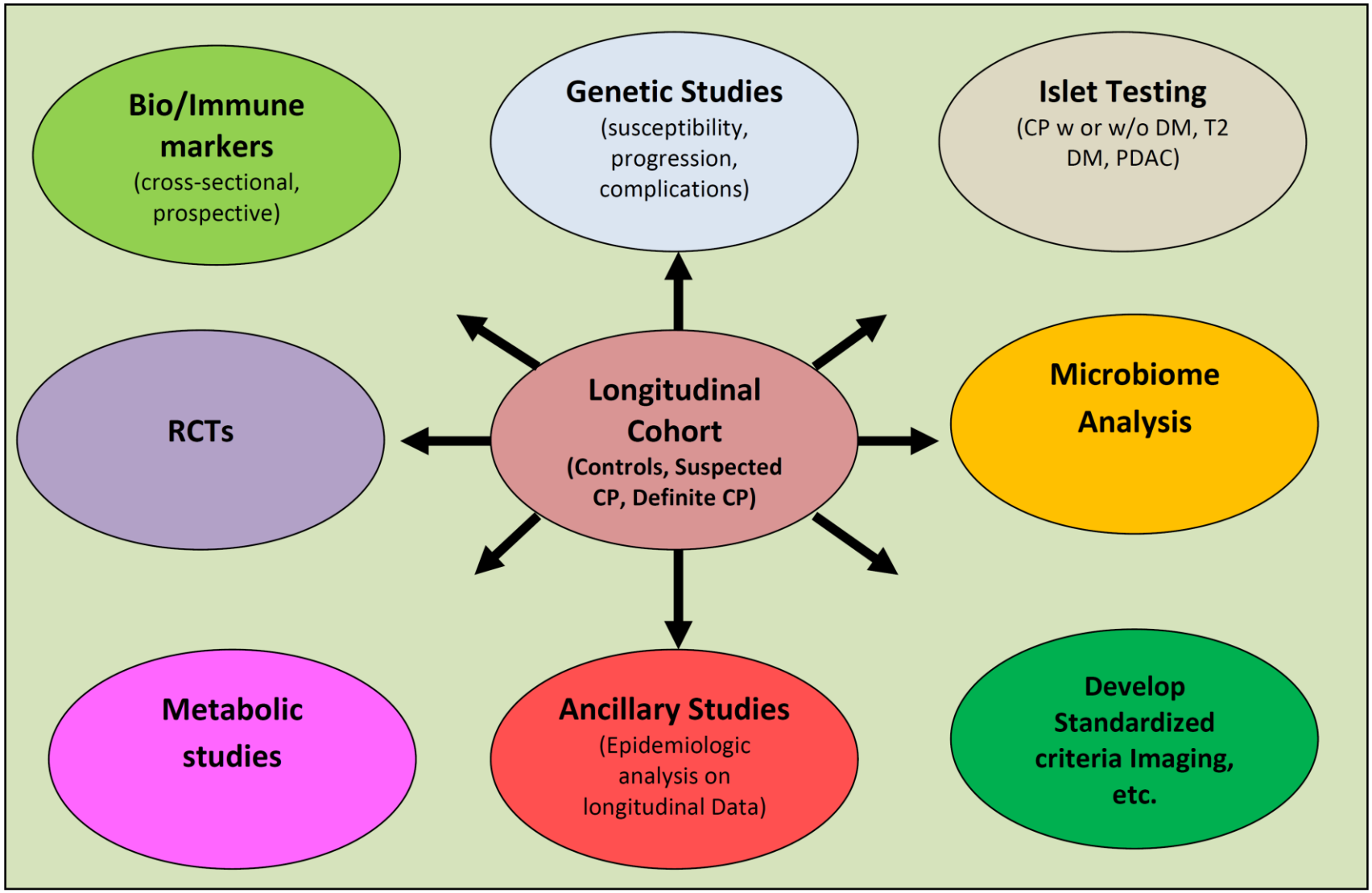
Yadav, D et al., Pancreas 2018.

Adult CP_RAP Scope of Work

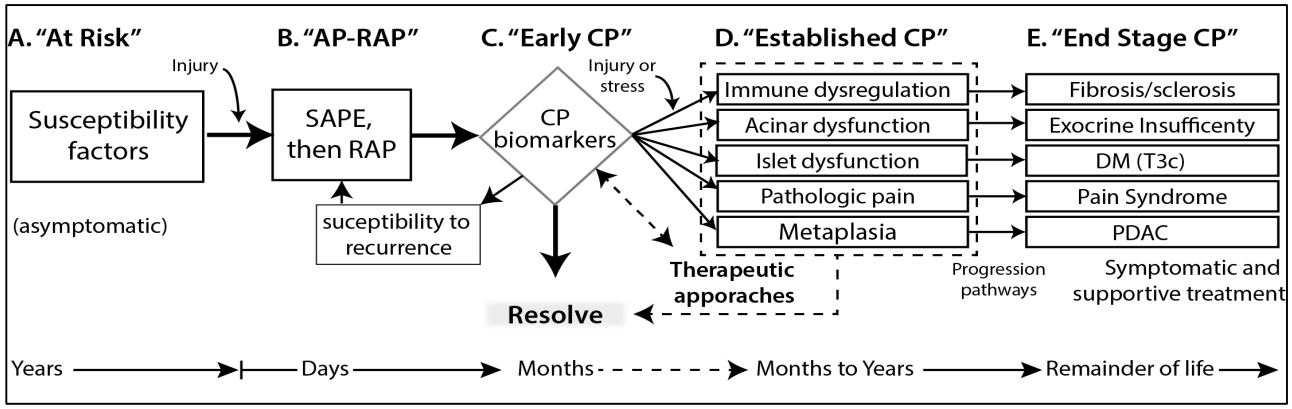
Four (4) Primary Objectives

1. **Establish** a model longitudinal research cohort
2. **Estimate** the risk of disease related complications
3. **Validate** predictive and diagnostic candidate biomarkers
4. **Develop** a biorepository platform to perform genetic and mechanistic studies

Adult CP_RAP Scope of Work

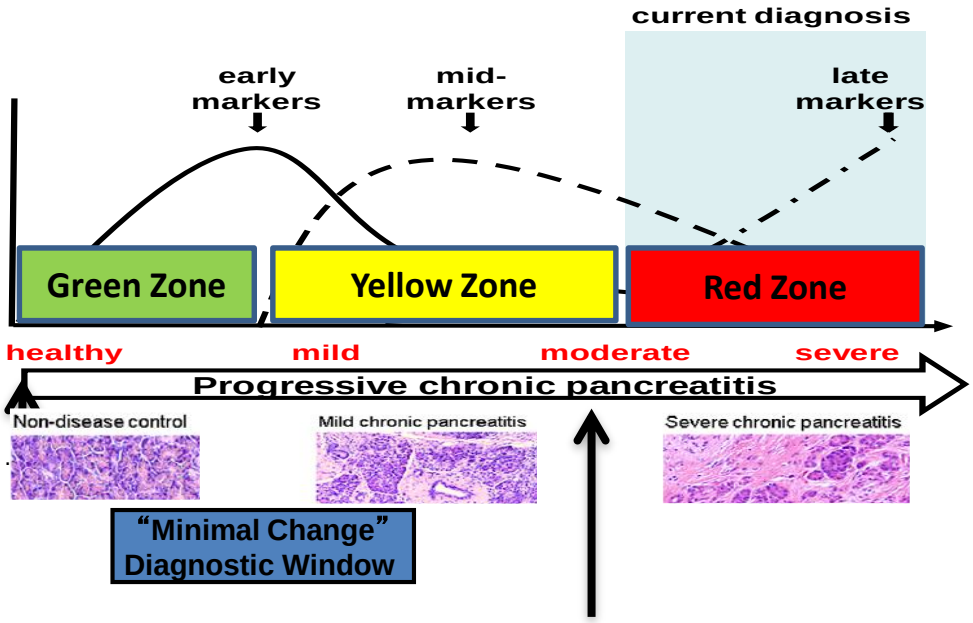


PROCEED STUDY



Early

Imaging / Biospecimens



Late

Imaging / Biospecimens

Proteins, RNA, DNA, cytokines, miRNA, lipids, microbiome

Adult Cohort Definitions

Cohort	CONTROLS		Suspected Chronic Pancreatitis		Chronic Pancreatitis
	Normal Volunteer	Suspected Pancreatic Origin	Recurrent Acute Pancreatitis	Indeterminate	Established
Minimal Inclusion Criteria	No symptoms **AND** No Risk Factors **AND** No Fam. History **AND** No DM **AND** Normal MRCP **AND** *Normal EUS (subset)	Abdominal pain **AND** No AP/CP **AND** Normal Cambridge **AND** ≤ 2 EUS Score **AND** No Sphincterotomy	At least 2 AP **AND** Normal Cambridge CT **AND** Normal MRCP **AND** ≤ 2 EUS Score **AND** Non-biliary Etiology	Clinical Presentation **AND** [Cambridge 1-2 or ≥ 3 EUS Score]	Cambridge ≥ 3 **OR** Abnormal Histology **OR** Parenchymal Calcifications
Longitudinal Follow-up	NO	YES	YES	YES	YES
Outcomes	N/A	RAP Chronic Pancreatitis	Chronic Pancreatitis	Chronic Pancreatitis	Exocrine Insufficiency Type 3cDM Cancer
Sample Size	100 (50)*	250 (100)	**660 (330)**		**660 (45)**
Clinical presentation	No symptoms	Pancreatic Type Pain	Recurrent Acute Pancreatitis (2 or more AP attacks)	AP (one or more) and/or Chronic pain	RAP ± Chronic pain or No symptoms
TIGAR-O CP Risk factors	-	±	±	±	±
Cambridge Imaging Grade (MRI/CT)	Normal	Normal	Normal	Grade I - II	Grade III - IV AND/OR Calcifications
EUS Score	0 - 2	0 - 2	0 - 2	≥3	≥5
Histology	Normal	Normal	Normal	Fibrosis (Ammann 1-6) and either inflammation and/or acinar cell loss	Fibrosis (Ammann 7-12) and Inflammation and Acinar cell loss

Standard Operating Procedures for Biospecimen Collection, Processing, and Storage

From the Consortium for the Study of Chronic Pancreatitis, Diabetes, and Pancreatic Cancer

William E. Fisher, MD, FACS, Zobeida Cruz-Monserrate, PhD,† Amy L. McElhany, MPH,* Gregory B. Lesinski, PhD,‡ Phil A. Hart, MD,† Ria Ghosh, MBA, MPH,§ George Van Buren, MD,* Douglas S. Fishman, MD,|| Jo Ann S. Rinaudo, PhD,¶ Jose Serrano, MD, PhD,# Sudhir Srivastava, PhD,¶ Thomas Mace, PhD,† Mark Topazian, MD,** Ziding Feng, PhD,§ Dhiraj Yadav, MD,†† Stephen J. Pandol, MD,‡‡ Steven J. Hughes, MD,§§ Robert Y. Liu, MS,|||| Emily Lu, MS,|||| Robert Orr, BS,¶¶ David C. Whitcomb, MD, PhD,** Amer S. Abouhamze, MHA,## Hanno Steen, PhD,*** Zachary M. Sellers, MD, PhD,††† David M. Troendle, MD,‡‡‡ Aliye Uc, MD,§§§ Mark E. Lowe, MD, PhD,|||||| and Darwin L. Conwell, MD,† on behalf of the Consortium for the Study of Chronic Pancreatitis, Diabetes, and Pancreatic Cancer (CPDPC)*

(*Pancreas* 2018;47: 1213–1221)

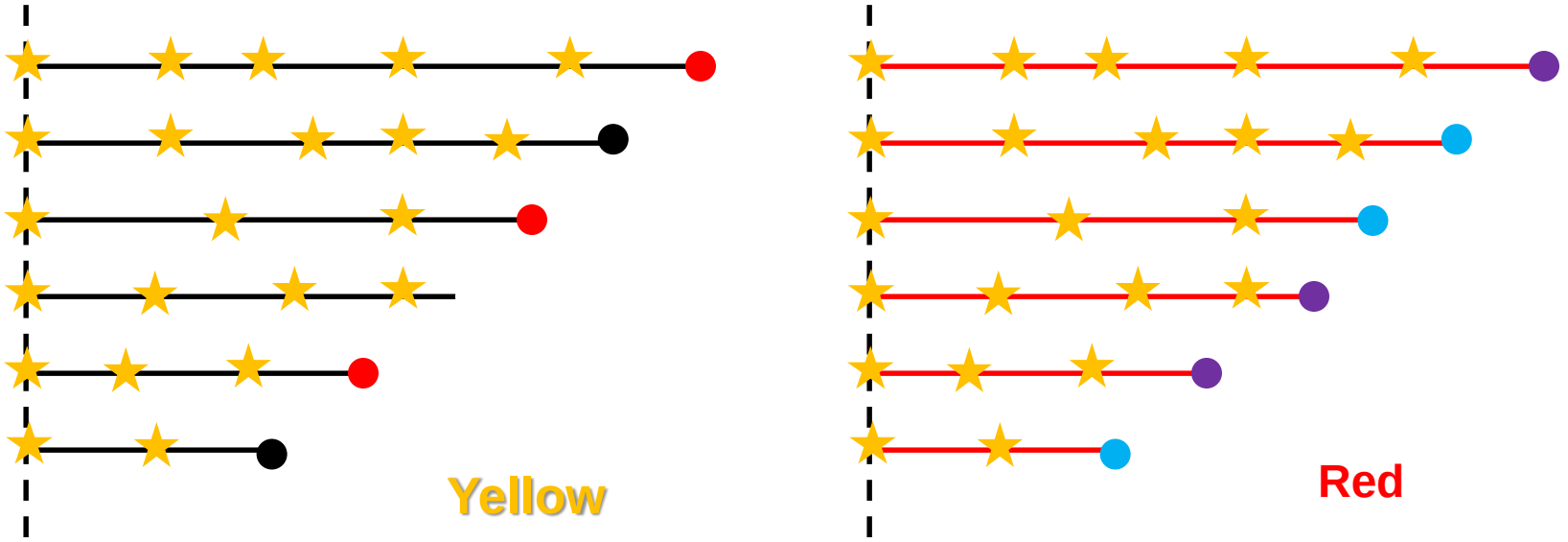
PROCEED Cohort Study

Prospective collection

- **Controls (250)**
- **Acute Pancreatitis (660)**
- **Chronic Pancreatitis (660)**

- Case Report forms
  - Patient, physician
- Labs
- Imaging: CT and MRI/MRCP and DEXA
- Endoscopic Ultrasound
- Biospecimens
  - Urine
  - Blood
  - Saliva
  - stool

PROCEED Cohort Study



Sub-cohort	Yellow (suspected CP)	Red (definite CP)
Endpoint	**Progression to definite CP**	**PDAC**, **new-onset DM**
Longitudinal data	AP/RAP, biomarkers, morphology, pain/symptoms, endocrine/exocrine pancreatic insufficiency	

Biomarker in Chronic Pancreatitis **(BioChiP)** Study: Urine & Pancreas Fluid

and

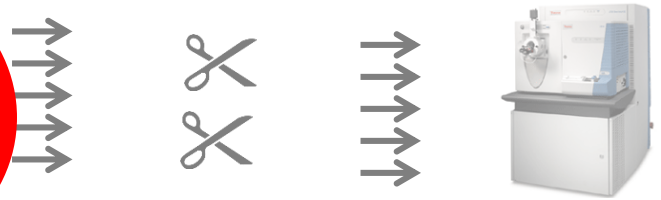
Magnetic Resonance Imaging as a Non-Invasive Method for the Assessment of Pancreatic Fibrosis **(MINIMAP)**

Optimizing the Urine Proteomics Pipeline

Technological Innovation and Resources

MStern Blotting–High Throughput Polyvinylidene Fluoride (PVDF) Membrane-Based Proteomic Sample Preparation for 96-Well Plates*

Sebastian T. Berger‡¶, Saima Ahmed‡¶, Jan Muntel‡¶, Nerea Cuevas Polo‡¶**, Richard Bachur§, Alex Kentsis‡‡, Judith Steen, and Hanno Steen‡¶§§



Journal of proteome research

Article

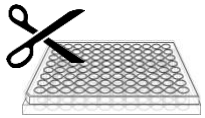
pubs.acs.org/jpr

Advancing Urinary Protein Biomarker Discovery by Data-Independent Acquisition on a Quadrupole-Orbitrap Mass Spectrometer

Jan Muntel,[†] Yue Xuan,[‡] Sebastian T. Berger,[†] Lukas Reiter,[§] Richard Bachur,[⊥] Alex Kentsis,[¶] and Hanno Steen*[,†]

New Strategy

150 *µl* urine



MStern blot

DIA
LC-MS

hours

Reduced sample needs:
3% of the original volume!

Reduced processing time:
Complete processing in 8 hrs!

No compromise in protein identifications!

(2) Candidate Urine Biomarker Proteins have also been identified: **U-BioCHiP Panel**

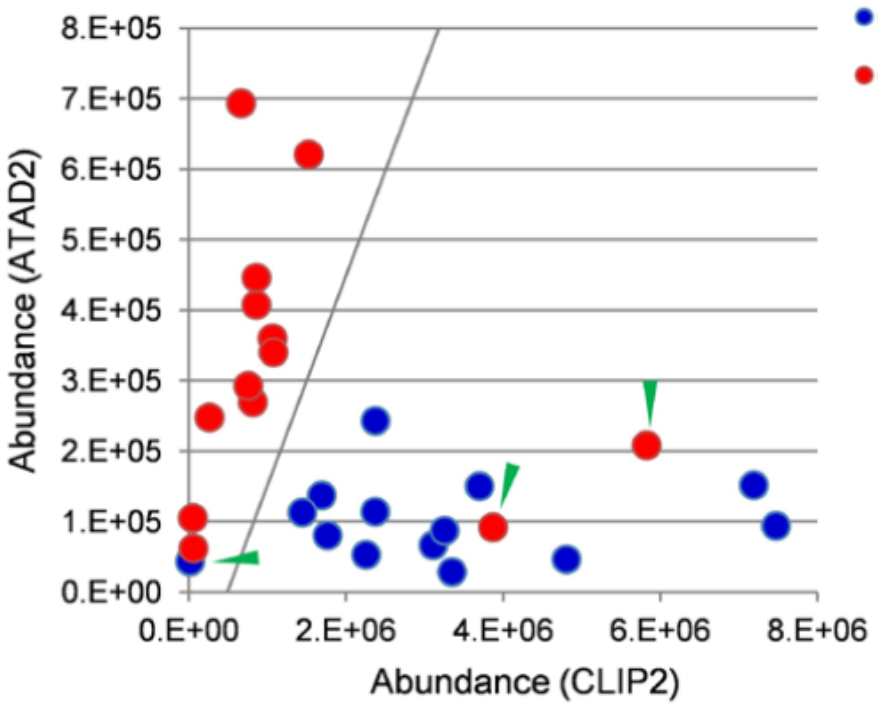
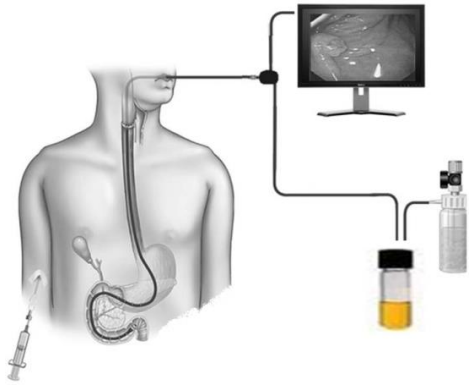


Figure 6. Random Forest-based classification of quantitative maps of urine samples from CP patients (bicarbonate <75 meq/L, red dots) and controls (bicarbonate >80meq/L, blue dots) identifies CLIP2 and ATAD2 as best classifiers. Xy-scatter plot for these two proteins. Green arrowheads indicate misclassified urine samples based on separation indicated by grey line.

AUC = 0.89

Endoscopic (ePFT) Collection of Pancreas Fluid

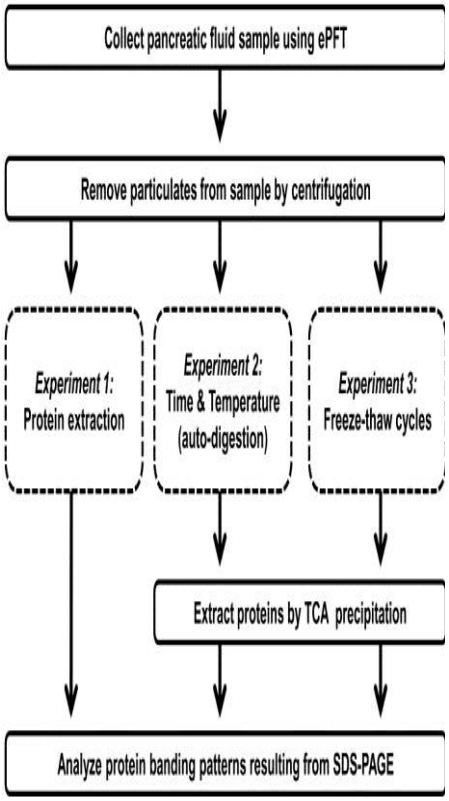
A "Bridge" to Translational Research



pancreatic fluid collection

Time (min) 0 15 30 45 60

secretin injection



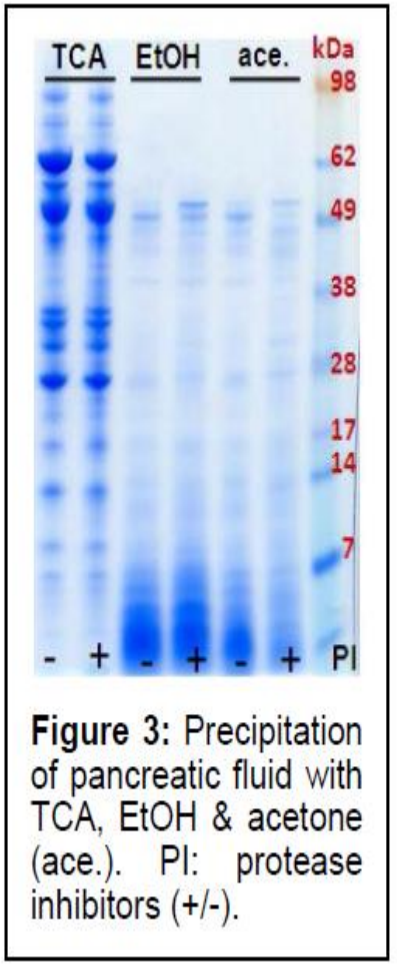


Figure 3: Precipitation of pancreatic fluid with TCA, EtOH & acetone (ace.). PI: protease inhibitors (+/-).

Paulo JA et al., Pancreas. 2010
Paulo JA et al., Electropheresis 2010
Paulo JA et al., Proteomics Clin Appl. 2010
Paulo JA et al., J Immunol Methods. 2011
Hart PA et al., Am J Gastro 2016

www.epft.net

5 - Candidate Pancreas Fluid Biomarker Proteins have been identified for CP: **P-BioChiP Panel**

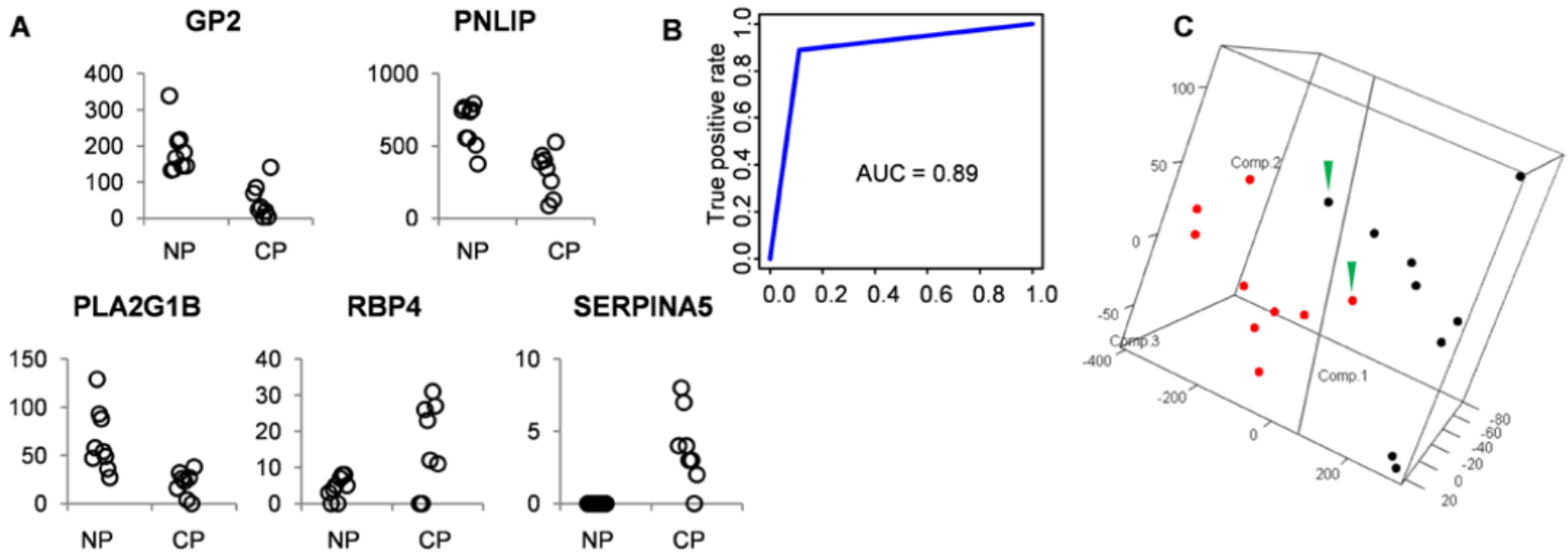
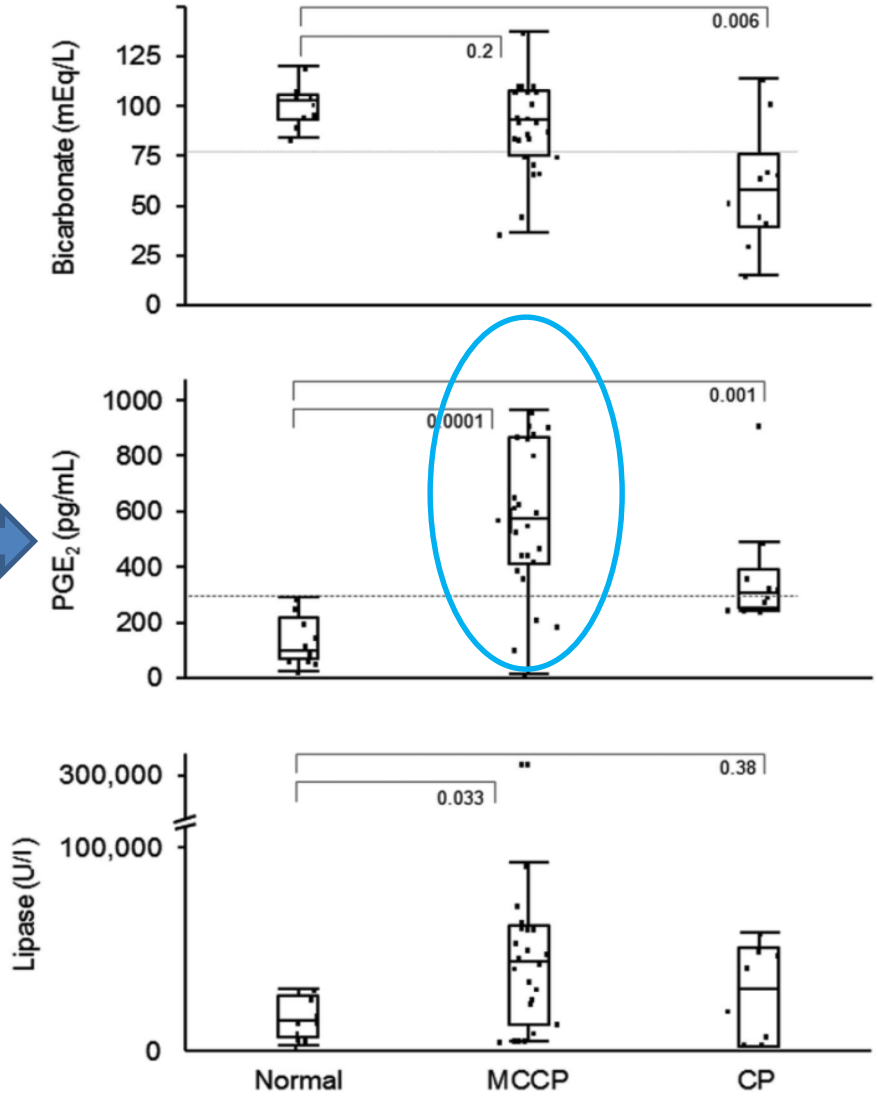


Figure 4. Random Forest-based classification of pancreatic fluid proteomes associated with CP patients and controls (NP). A) Scatter plots of the members of the protein panel with the best classification performance. B) Resulting ROC curve with an AUC of 0.89. C) Principal Component Analysis of the 18 pancreatic fluid specimens based on the 5 protein biomarker panel; red: CP; blue: NP. Green arrowheads indicate misclassified samples.

AUC = 0.89

Pancreatic Juice Prostaglandin E2 Concentrations Are Elevated in Chronic Pancreatitis and Improve Detection of Early Disease

Barham K. Abu Dayyeh, MD[1], Darwin Conwell, MD, MS[2], Navtej S. Buttar, MD[1], Vivek Kadilaya, MD[2], Philip A. Hart, MD[1], Nikola A. Baumann, PhD[3], Benjamin L. Bick, MD[1], Suresh T. Chari, MD[1], Sonia Chowdhary, MD[1], Jonathan E. Clain, MD[1], Ferga C. Gleeson, MD[1], Linda S. Lee, MD[2], Michael J. Levy, MD[1], Randall K. Pearson, MD[1], Bret T. Petersen, MD[1], Elizabeth Rajan, MD[1], Hanno Steen, PhD[4], Shadeah Suleiman, BS[2], Peter A. Banks, MD[2], Santhi S. Vege, MD[1] and Mark Topazian, MD[1]



AUC = 0.89

Conclusion:

1. PJ PGE2 concentrations are elevated in CP and MCCP

2. PJ PGE2 concentration may be useful diagnostically

3. **In addition, our findings support the concept that COX-2 inhibition might modify disease progression at early stages.**

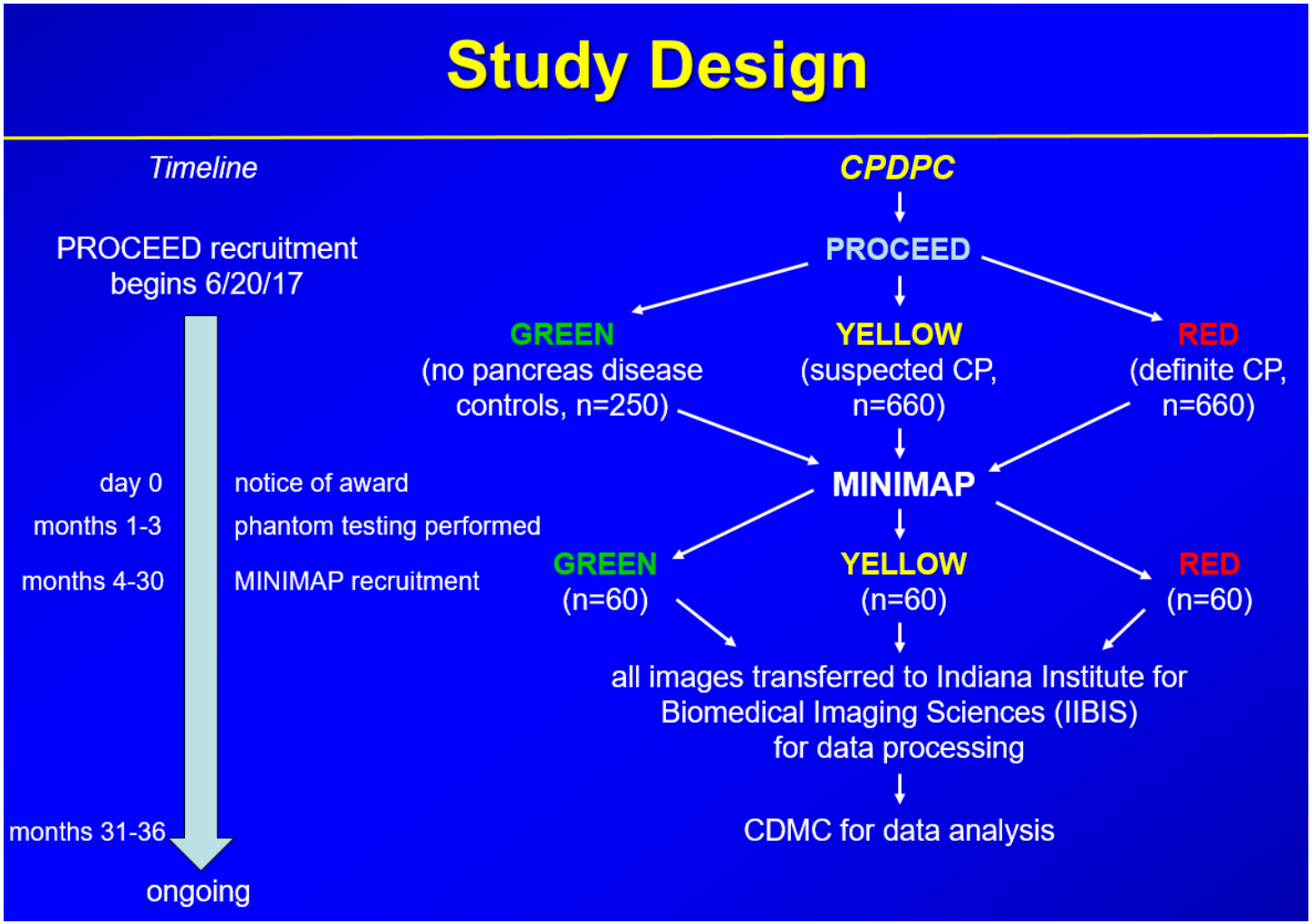
PAIR STUDY
-preliminary data

Abu Dayyeh, BK et., Clin Transl Gastro 2015

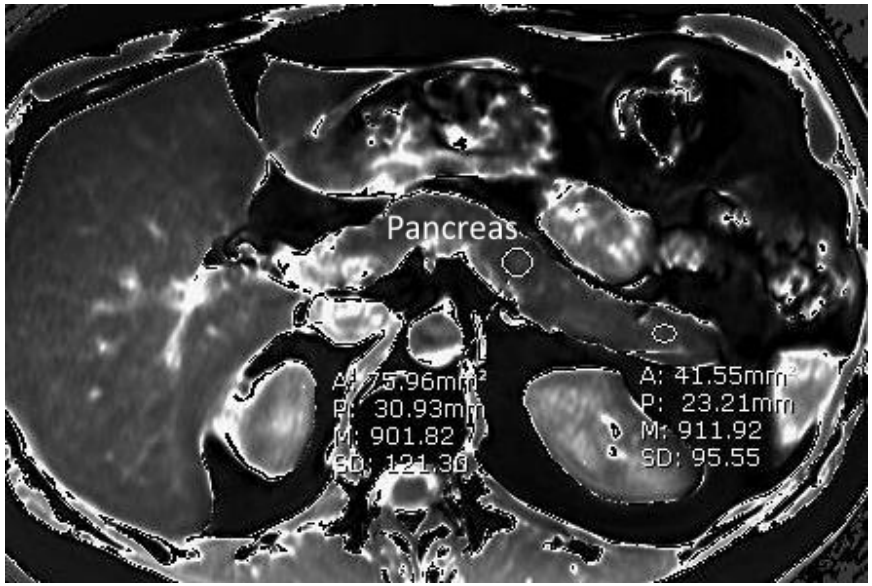
Magnetic Resonance Imaging as a Non- Invasive Method for the Assessment of Pancreatic Fibrosis **(MINIMAP)**

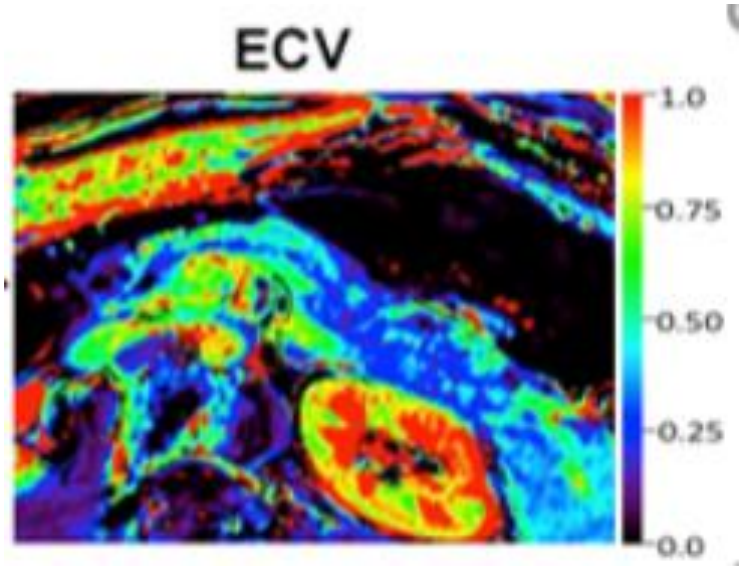
Evan L. Fogel, M.D. and Temel Tirkes, MD
Indiana University Hospital
Indianapolis, Indiana

Magnetic Resonance Imaging as a Non- Invasive Method for the Assessment of Pancreatic Fibrosis **(MINIMAP)**



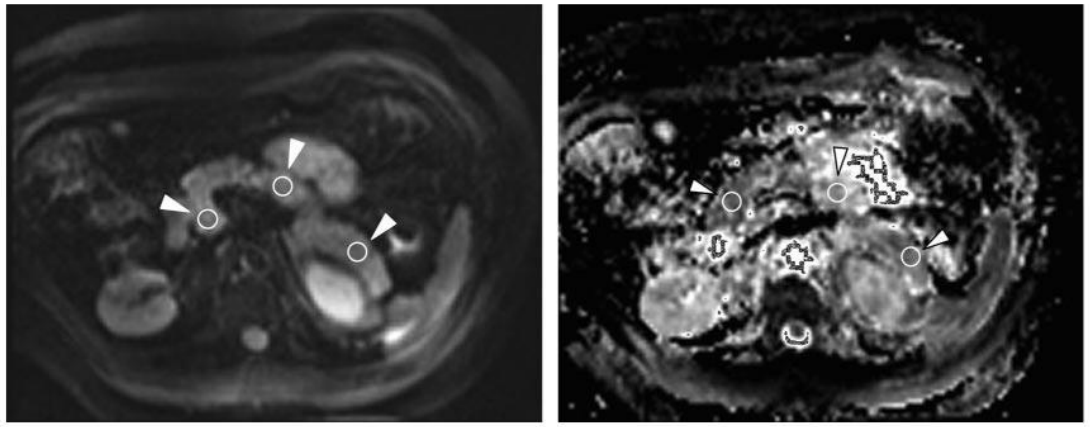
T1 mapping


Pancreas
A: 75.96mm²
P: 30.93mm
M: 901.82
SD: 121.30
A: 41.55mm²
P: 23.21mm
M: 911.92
SD: 95.55


ECV
1.0
0.75
0.50
0.25
0.0

Diffusion-Weighted Imaging

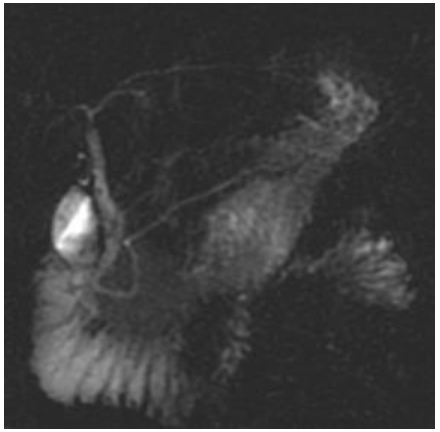
- Measures free motion of water molecules within the tissues
- More time-consuming: 5-7 minutes to perform



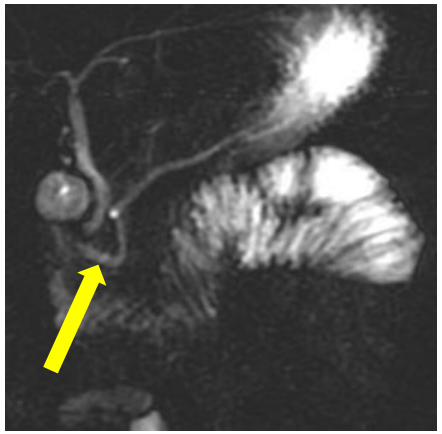
a. b.

Secretin-stimulated MRCP

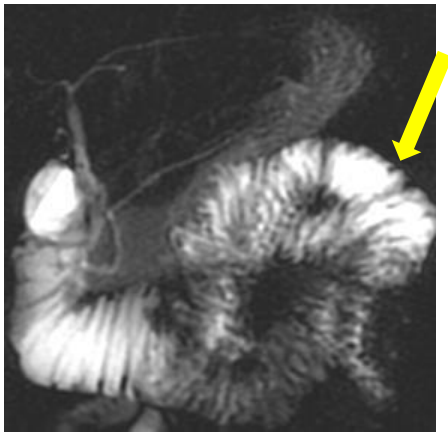
- Time: 10-12 minutes
- Provides information about ductal anatomy as well as exocrine volume, function of the pancreas



2D thick slab MRCP
before secretin



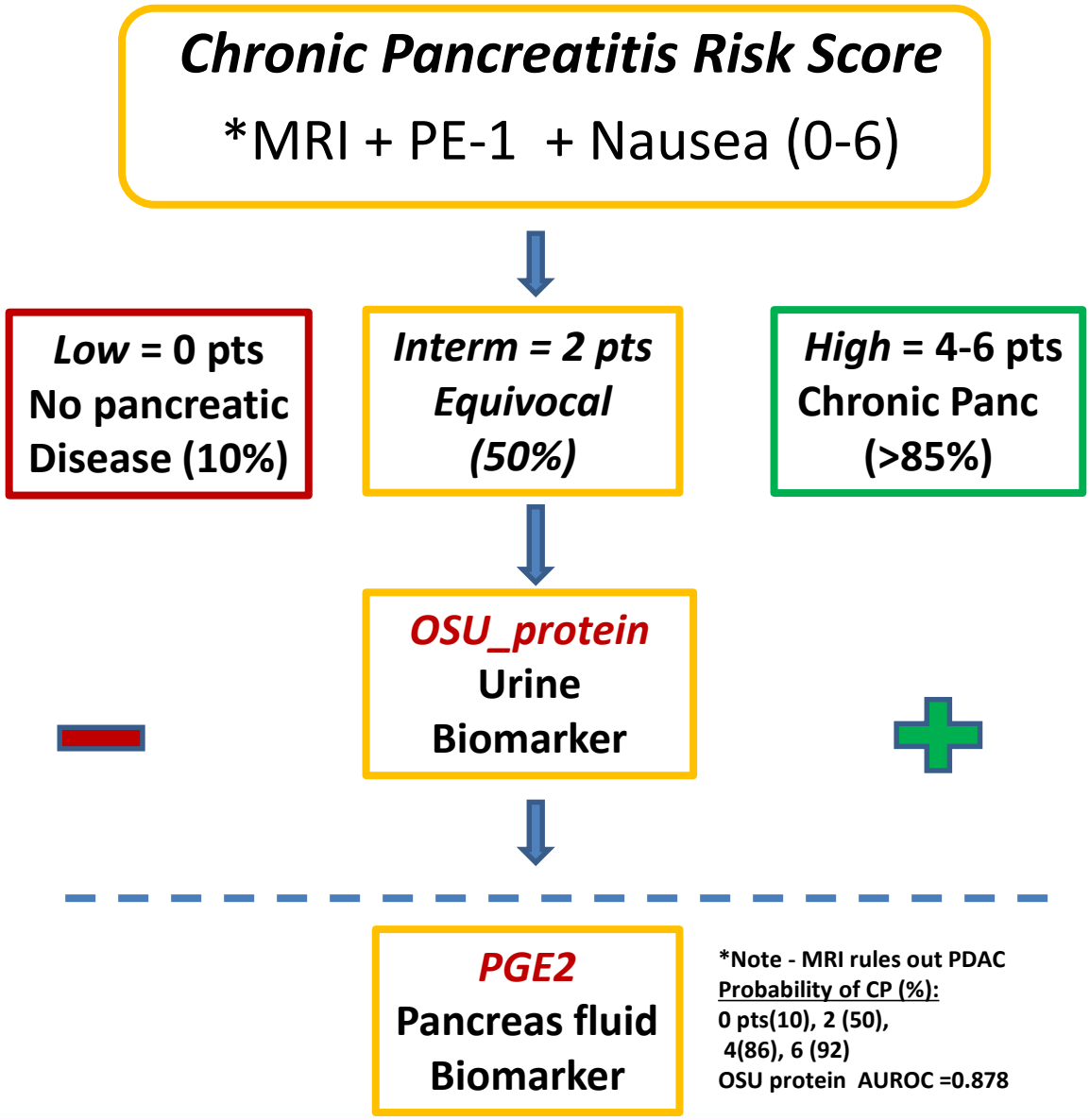
increased caliber of
pancreatic duct, 2min
after secretin



return to baseline of PD
15 minutes after secretin, with increased
duodenal fluid

THE OHIO STATE UNIVERSITY
WEXNER MEDICAL CENTER

Future CP Diagnosis Algorithm



Non-invasive

-urine

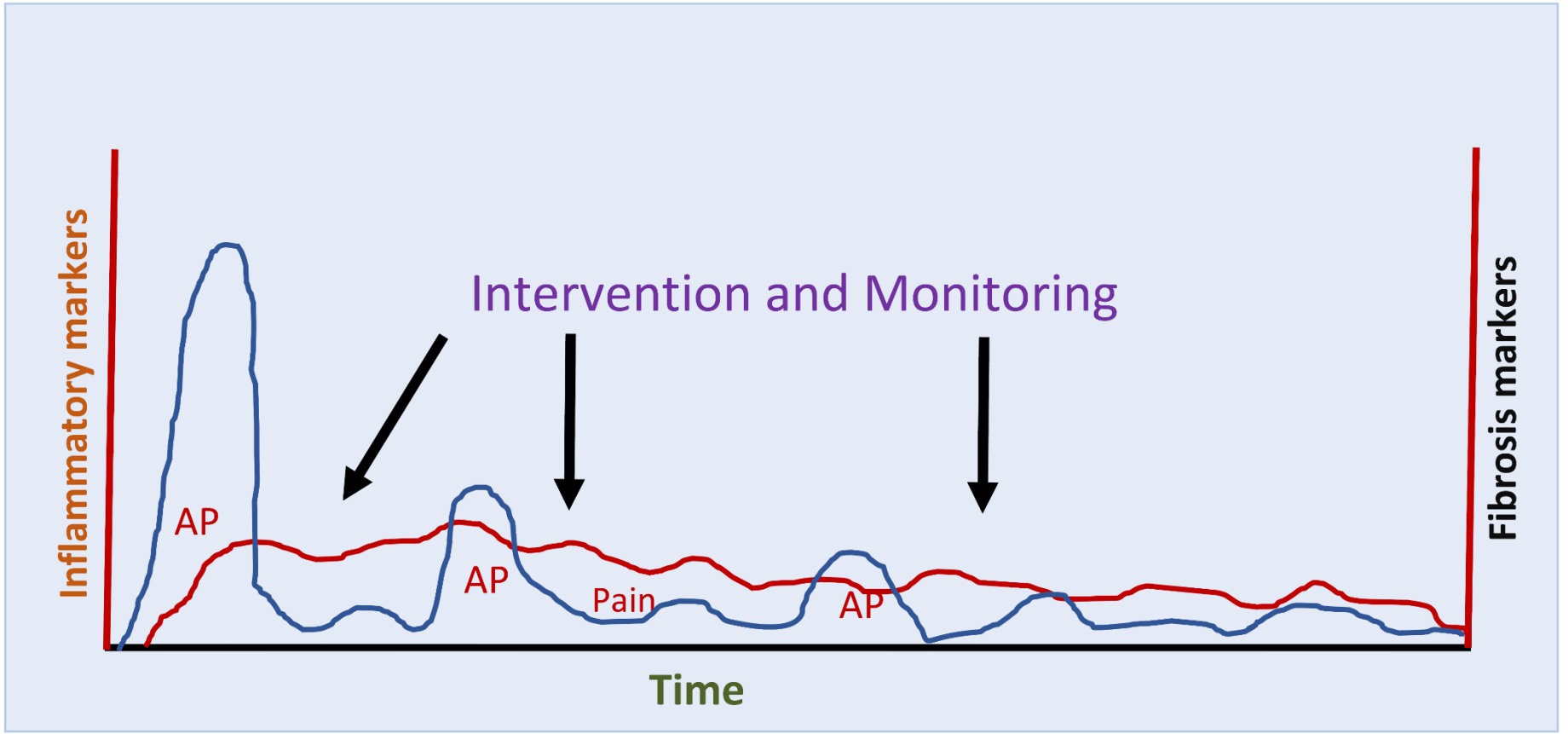
-UBioChiP

Invasive

-pancreas fluid

-PBioChiP

Conceptual framework – CP



Modified from: Colombel J et al. Gastroenterology 2017;152:351-61

Conclusion

- **PROCEED - Prospective Longitudinal Cohort**
  - Natural History of RAP to CP
  - Natural History of CP and sequelae
  - Framework for Biomarker Discovery and Validation
  - Multicenter Collaboration


NIH
National Institute of
Diabetes and Digestive
and Kidney Diseases


NATIONAL
CANCER
INSTITUTE


KAISER
PERMANENTE
THE OHIO STATE UNIVERSITY
WEXNER MEDICAL CENTER
UNIVERSITY OF IOWA
HOSPITALS & CLINICS
University of Iowa Health Care
CEDARS-SINAI
UFHealth
UNIVERSITY OF FLORIDA HEALTH
HEALTH
Indiana University Health
Stanford
MEDICINE
Baylor
College of
Medicine
MAYO
CLINIC
University of
Pittsburgh