



# LOCAL INFLAMMATION IN BREAST TISSUE AND MAMMOGRAPHIC DENSITY AMONG PREMENOPAUSAL AND POSTMENOPAUSAL WOMEN

**Mirette Hanna**

MD – Clinical pathology

PhD (candidate) – Experimental medicine

MSc – Clinical and chemical pathology

Faculty of Medicine - Laval University

Quebec - Canada



Immunology summit

2014

# **Disclosure of potential conflicts of interest**

No conflict of interest to declare

# Outlines

- Introduction
  - Inflammation and cancer
  - Breast cancer risk factor
- Objective
- Materials and Methods
  - Study population
  - Assessment of inflammatory markers
  - Assessment of mammographic density
  - Statistical analyses
- Results
- Conclusions and perspectives
- Acknowledgements

# Introduction

## Inflammation and cancer



Review

### Inflammation and cancer: back to Virchow?

Fran Balkwill, Alberto Mantovani

The response of the body to a cancer is not a unique mechanism but has many parallels with Inflammation and wound healing. This article reviews the links between cancer and inflammation and discusses the implications of these links for

cancer  
to c  
tum  
infla  
canc  
that  
deve  
thes  
prov  
infla

NATIONAL INSTITUTES OF HEALTH  
NIH Public Access  
Author Manuscript  
*Nature*. Author manuscript; available in PMC 2010 January 7.

Published in final edited form as:  
*Nature*. 2002 December 19; 420(6917): 860–867. doi:10.1038/nature01322.

### Inflammation and cancer

Lisa M. C  
Lisa M. Cou  
\* Cancer  
† Departm  
‡ Departm  
§ UCSF C  
USA



Available online at [www.sciencedirect.com](http://www.sciencedirect.com)



Cytokine & Growth Factor Reviews 17 (2006) 325–337

Cytokine  
Growth Factor  
Reviews

[www.elsevier.com/locate/cytogfr](http://www.elsevier.com/locate/cytogfr)

Mini review

### Cytokines in breast cancer

A. Nicolini<sup>a,\*</sup>, A. Carpi<sup>b</sup>, G. Rossi<sup>c</sup>

### Chronic Inflammation and Breast Pathology: A Theoretical Model

Diana Lithgow and Chandice Covington

*Biol Res Nurs* 2005 7: 118

DOI: 10.1177/1099800405280823

The online version of this article can be found at:  
<http://bm.sagepub.com/content/7/2/118>

NIH-PA Author Manuscript

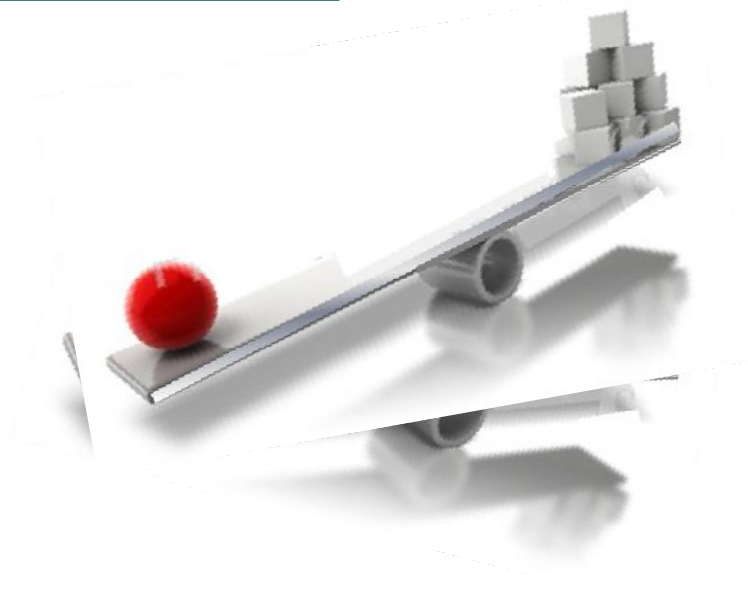
# Inflammation and cancer

## Pro-inflammatory markers

IL-6  
TNF- $\alpha$

## Anti-inflammatory markers

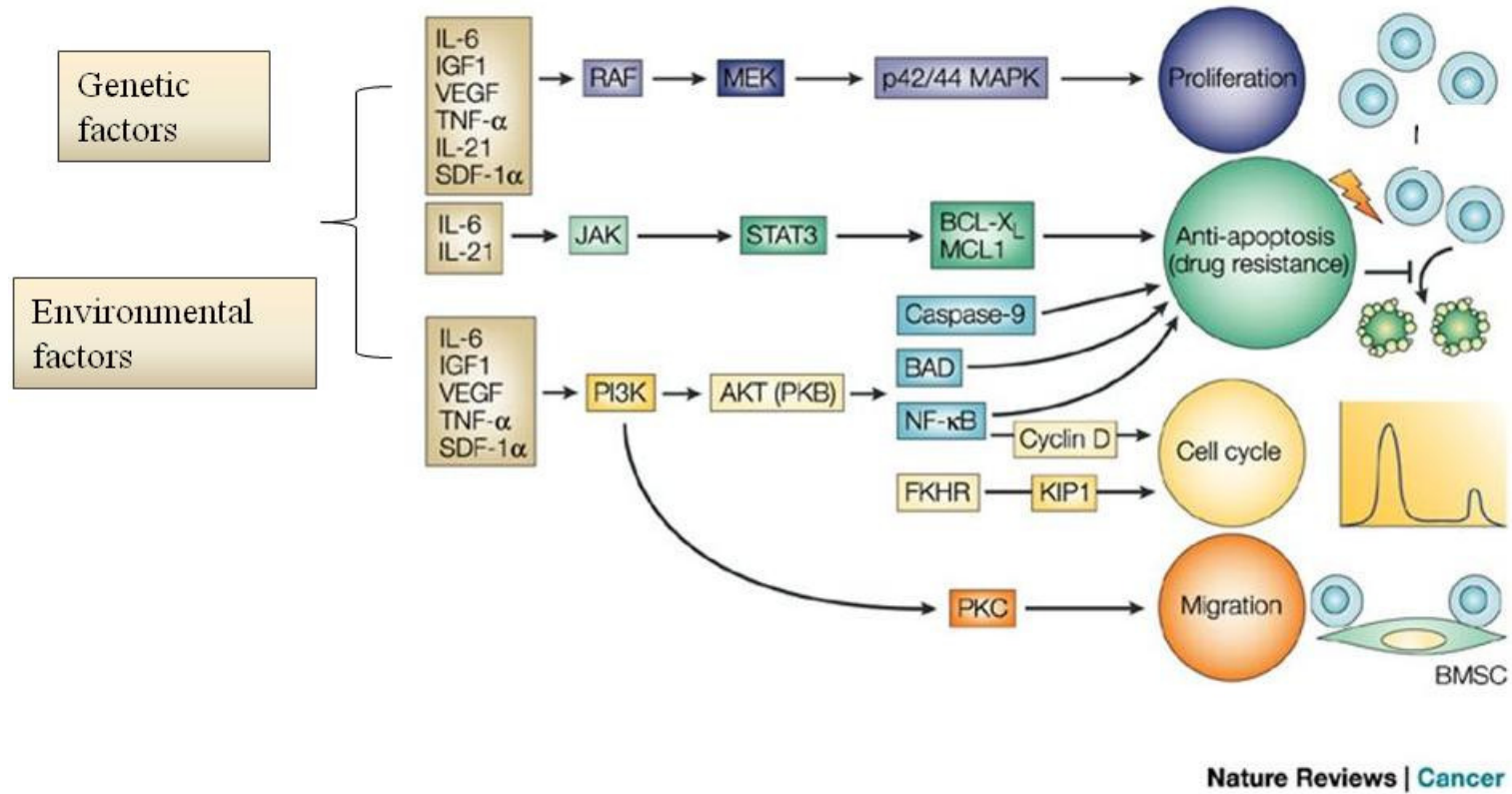
TGF- $\beta$



---

IL-6, interleukin 6; TNF- $\alpha$ , tumor necrosis factor- $\alpha$ ; TGF- $\beta$ , transforming growth factor- $\beta$

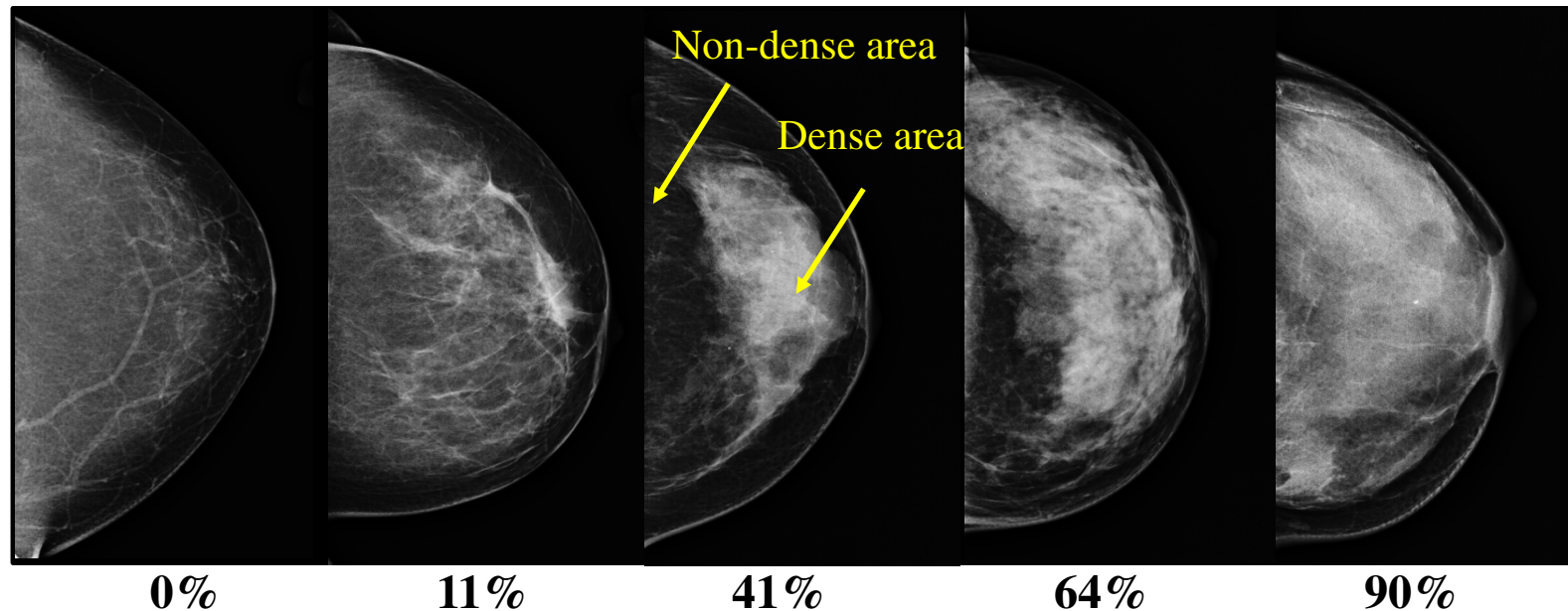
# Inflammation and cancer



IL-6, interleukin 6; TNF- $\alpha$ , tumor necrosis factor- $\alpha$

# Breast cancer risk factor

## Mammographic density

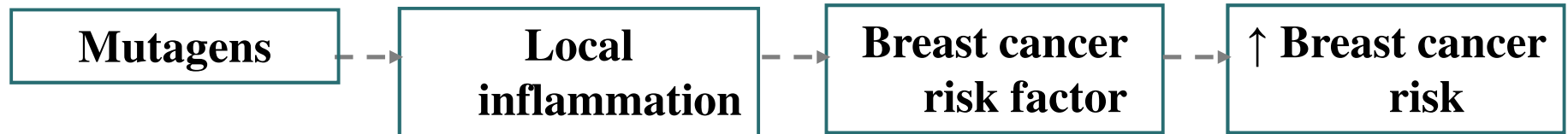


- Proportion of the breast occupied by fibroglandular tissue
- Proliferation of mammary epithelium and stroma induced by the cumulative exposure to growth factors and hormones

➔ Positively associated to breast cancer risk

*(Hanna et al., InTec Mammography-recent advances 2012; 9:173-198)*

# Hypothesis



- Genetic
- Environmental
- Life style

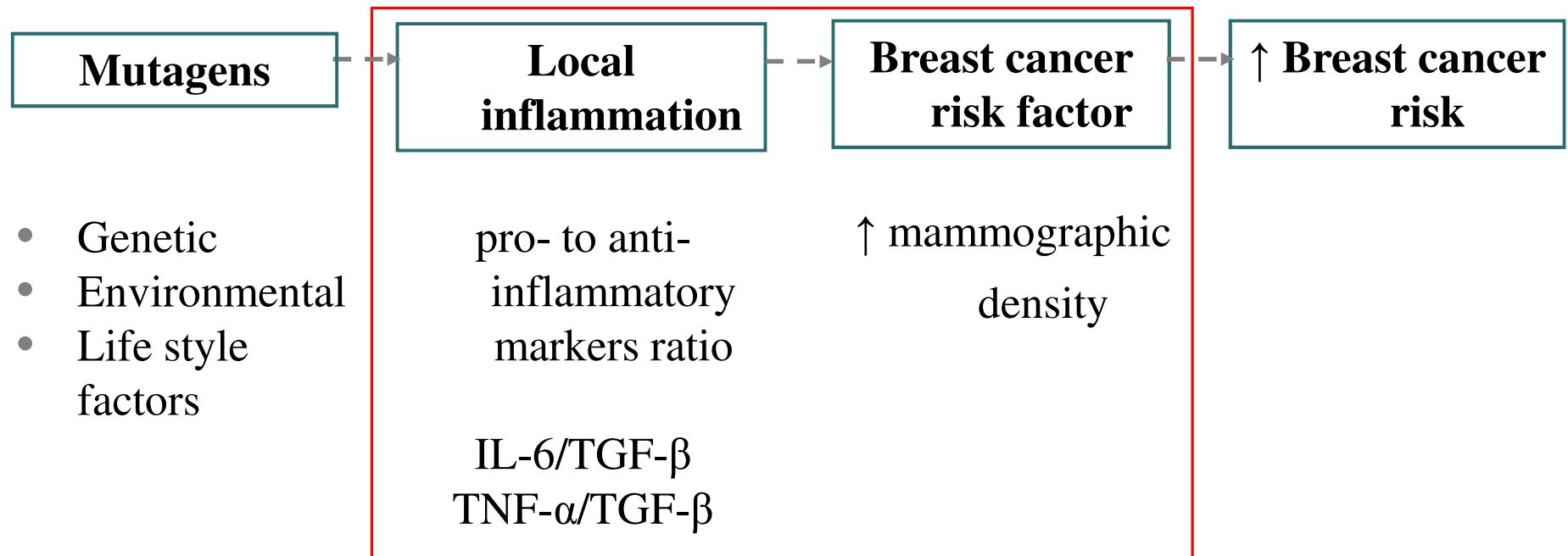
- ↑ pro-inflammatory markers

- ↓ anti-inflammatory markers

- ↑ mammographic density



# Objective



To evaluate the association between the pro- to anti-inflammatory markers ratio and the mammographic density.

# Materials and methods

## Study population

163 women diagnosed with breast cancer

### **Inclusion criteria**

<70 years

Mammography

No chemotherapy or radiotherapy

No breast surgery (reduction, augmentation or implant)

No history of other cancers

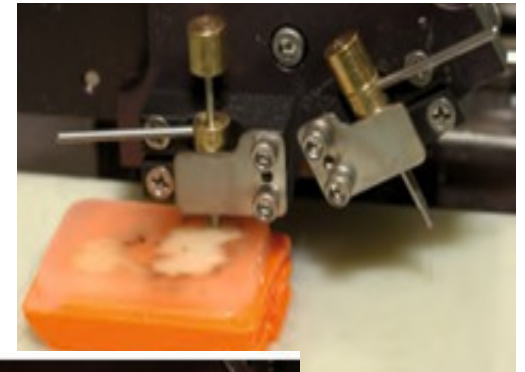
Not currently pregnant

# Inflammatory markers assessment

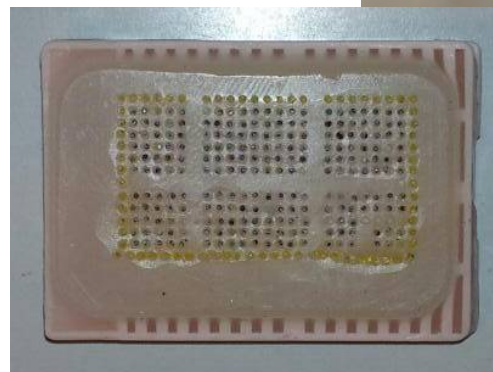
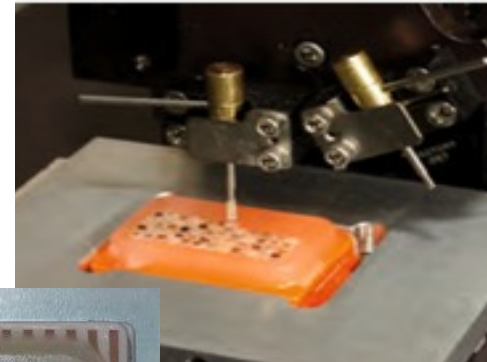
## A- Tissue microarray (TMA) construction

6 cores (1mm in diameter) of normal tissue/ patient

*(Beecher Instruments®Tissue Microarray Technology, Estigen, Sun Prairie, WI, USA)*



Control tissues (MCF-7, MDA-231 and SKBR-3)



# Inflammatory markers assessment

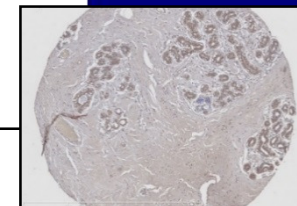
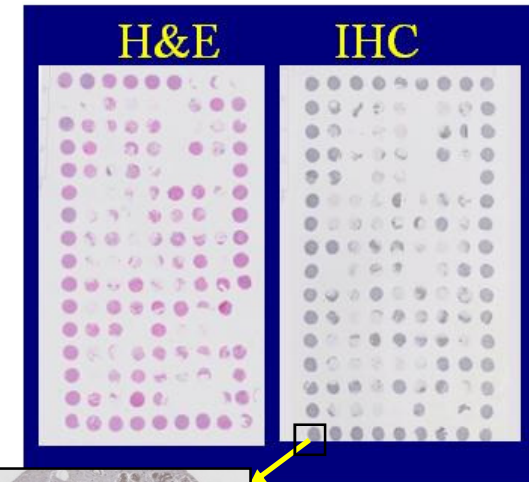
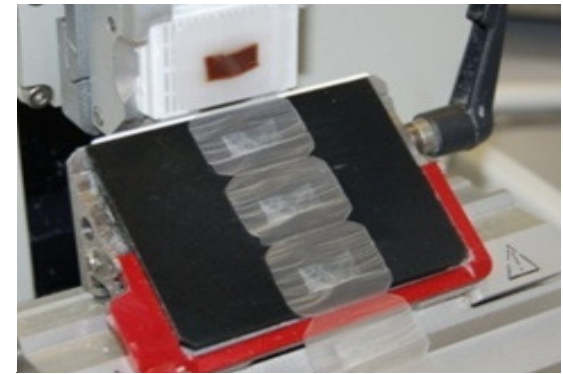
## B- Immunohistochemistry (IHC) staining

Serial sections (4 microns)

Coloration by H&E and immunohistochemistry

Positive and negative control in each cycle of staining

Scanning of TMA stained slides (*NanoZoomer 2.0HT, Hamamatsu*)



H&E, hematoxylin-eosin; TMA, tissue microarray

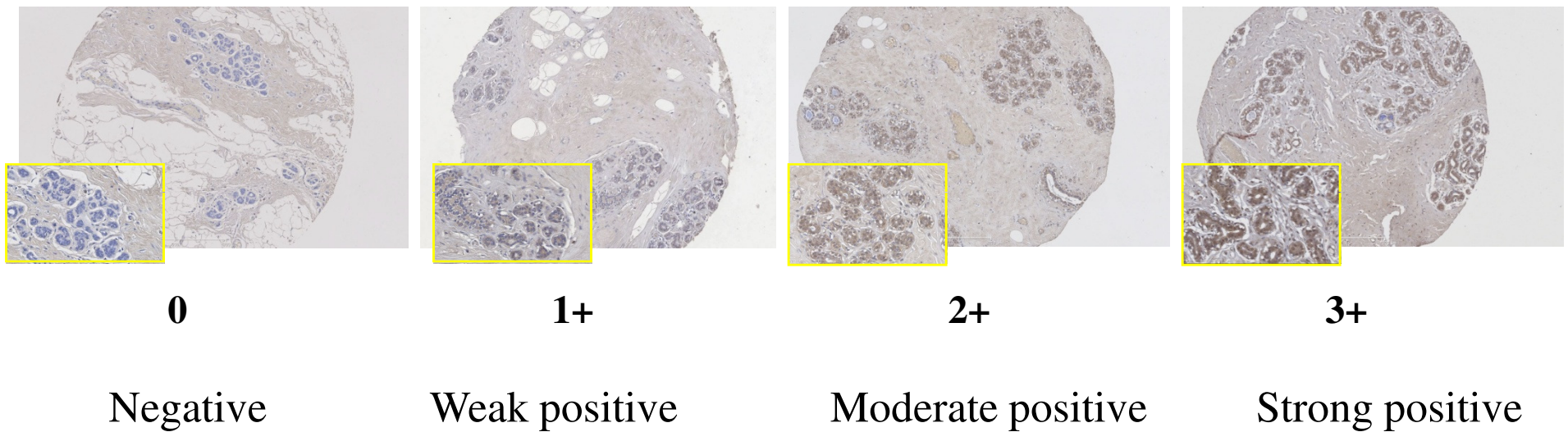
# Assessment of the expression of inflammatory markers in normal mammary epithelium

- Visual assessment
- One blinded reader
- Good concordance between quantitative analysis and visual estimation (Kappa >0.88)

*(Turbin et al., Breast Cancer Res Treat 2008, 110:417-26)*

# Assessment of the expression of inflammatory markers in normal mammary epithelium

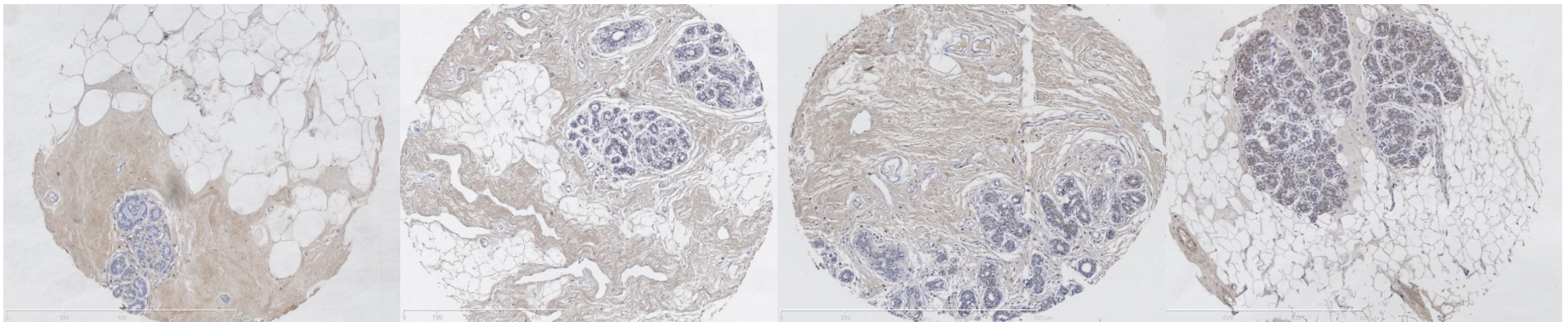
## 1. Intensity of immunostaining



**IL-6** (*IL6* (1): *sc-130326*; Santa Cruz Biotechnology)

# Assessment of the expression of inflammatory markers in normal mammary epithelium

## 2. Extend of immunostaining



**0**

**(0%)**

**1+**

**(1-9%)**

**2+**

**(10-50%)**

**3+**

**(>50%)**

Proportion of positively stained epithelial cells for IL-6 (*IL6 (1): sc-130326; Santa Cruz Biotechnology*)

# Assessment of the expression of inflammatory markers in normal mammary epithelium

Intensity of immunostaining (0-3)

X

Extend of immunostaining (0-3)

=

**Quick score**  
(0-9)



# Assessment of the expression of inflammatory markers in normal mammary epithelium

## Reproducibility of the assessment

- 5 randomly selected TMAs

K intra-observer = 0.75 (95% CI= 0.64-0.86)

K inter-observer = 0.74 (95% CI= 0.63-0.84)

# Assessment of the expression of inflammatory markers in normal mammary epithelium

Pro- to anti-inflammatory markers ratio

IL-6/TGF- $\beta$

TNF- $\alpha$ /TGF- $\beta$



Anti-inflammatory  
state

IL-6 < TGF- $\beta$   
TNF- $\alpha$  < TGF- $\beta$

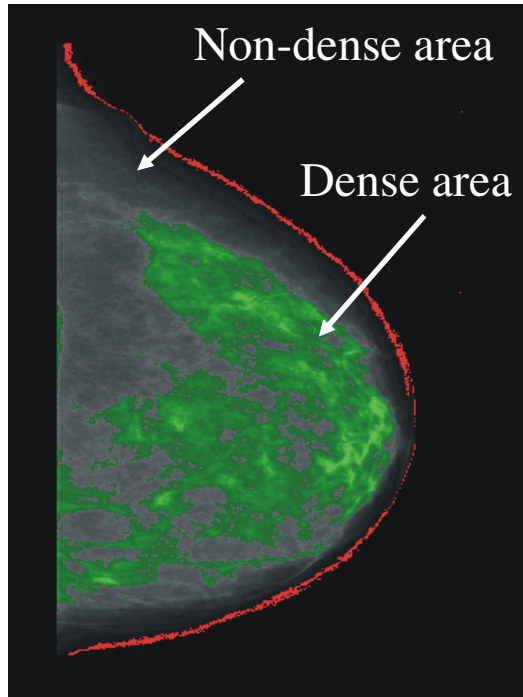
Neutral  
state

IL-6 = TGF- $\beta$   
TNF- $\alpha$  = TGF- $\beta$

Pro-inflammatory  
state

IL-6 > TGF- $\beta$   
TNF- $\alpha$  > TGF- $\beta$

# Mammographic density assessment



- Computer-assisted methods
- Non-affected breast
- Percent mammographic density  
$$= \frac{\text{number of pixels in dense breast area}}{\text{number of pixels in the whole breast}} \times 100$$
- Reproducibility

Correlation coefficient = 0.94

# Statistical analyses

- Generalized linear models
- Adjustment for potentially confounding factors
- Analyses stratified by menopausal status

# Results

## Characteristics of the study population

	All women (n = 163)	
Characteristic	Mean	SD
Age at breast surgery (years)	52.2	7.8
Body mass index (kg/m <sup>2</sup> )	27.0	5.7
Waist circumference (cm)	86.9	12.7
Age at first full-term pregnancy (years)	25.9	4.1
Alcohol consumption (drink/week)	4.3	4.6
Percent mammographic density (%)	22.5	14.7
	N	%
Postmenopausal	81	49.7
Parity	119	73.0
Breastfeeding	62	38.0
Oral contraceptives use	156	95.7
Hormone replacement therapy	54	33.1
Family history of breast cancer	34	20.9
Former or current smoker	94	57.7

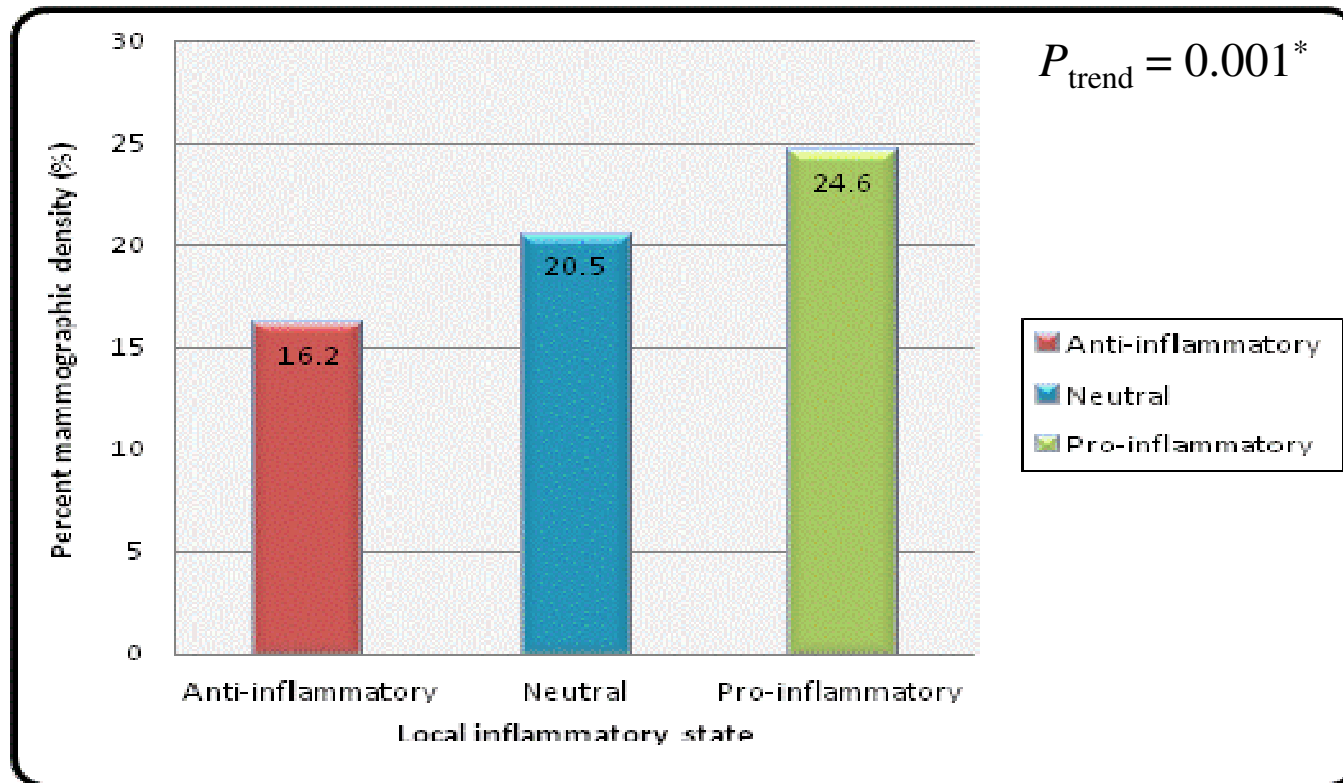
SD, Standard deviation

# Characteristics of the study population

Pro- to anti-inflammatory markers ratio	All women (n = 163)	
	N	%
IL-6/TGF- $\beta$		
Anti-inflammatory	34	20.9
Neutral	38	23.3
Pro-inflammatory	86	52.8
TNF- $\alpha$ /TGF- $\beta$		
Anti-inflammatory	42	25.8
Neutral	40	24.5
Pro-inflammatory	76	46.6

# Association between the expression of inflammatory markers and the percent mammographic density

## IL-6/TGF- $\beta$



\*Associations adjusted for age, waist circumference and menopausal status

Further adjustment did not change the results

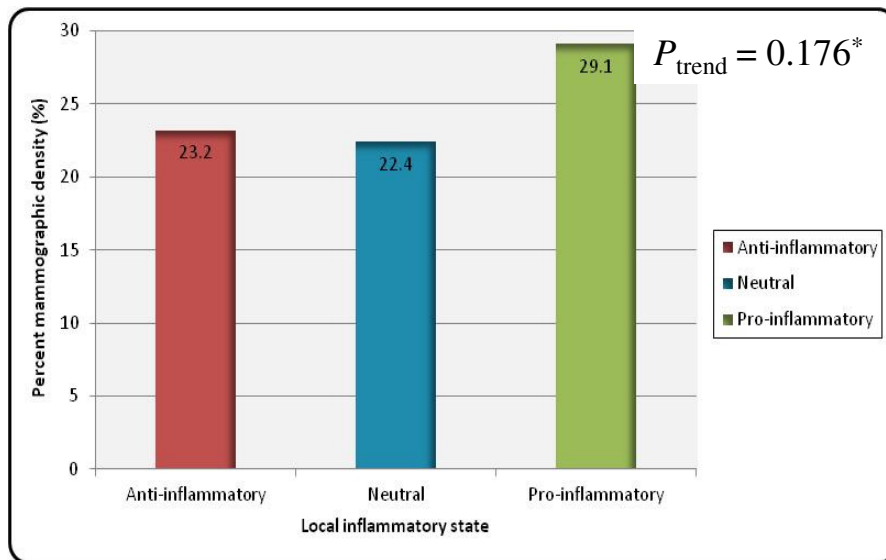


# Association between the expression of inflammatory markers and the percent mammographic density

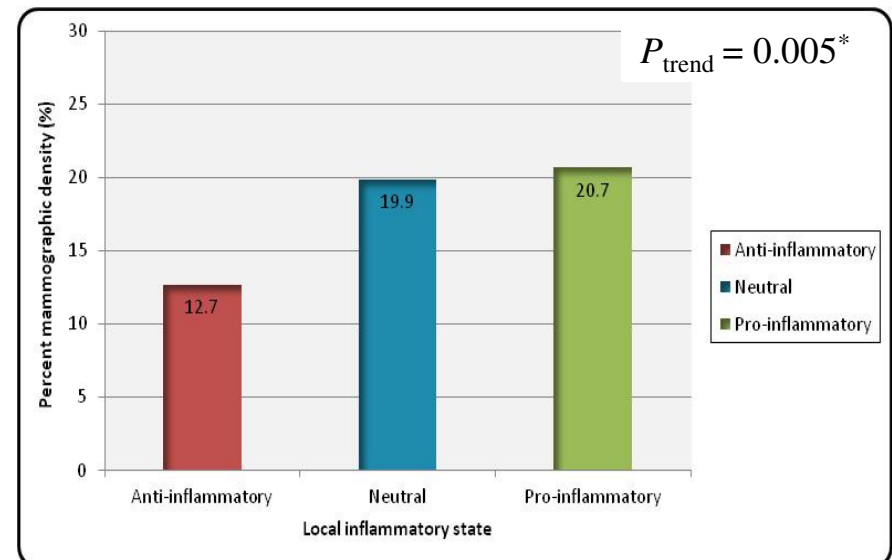
## IL-6/TGF- $\beta$

Analyses stratified by menopausal status

### Premenopausal women



### Postmenopausal women

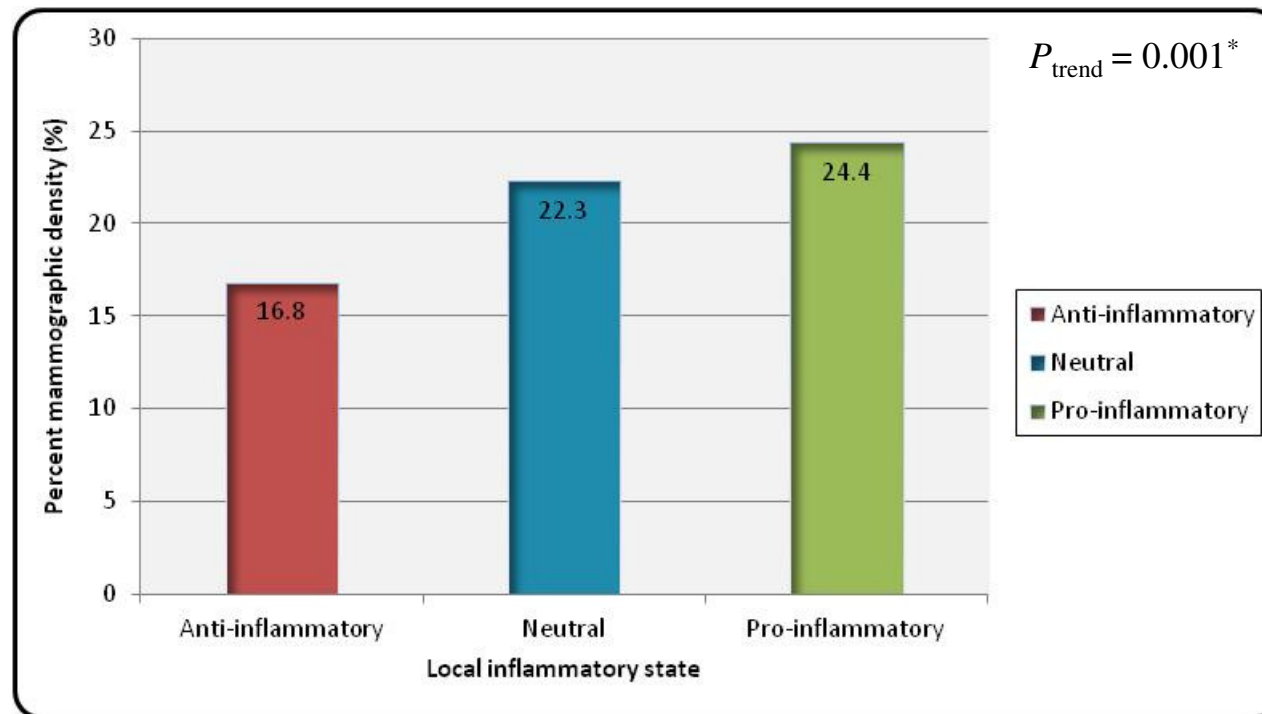


\*Associations adjusted for age and waist circumference

Further adjustment did not change any of the results

# Association between the expression of inflammatory markers and the percent mammographic density

TNF- $\alpha$ /TGF- $\beta$



\*Associations adjusted for age, waist circumference and menopausal status

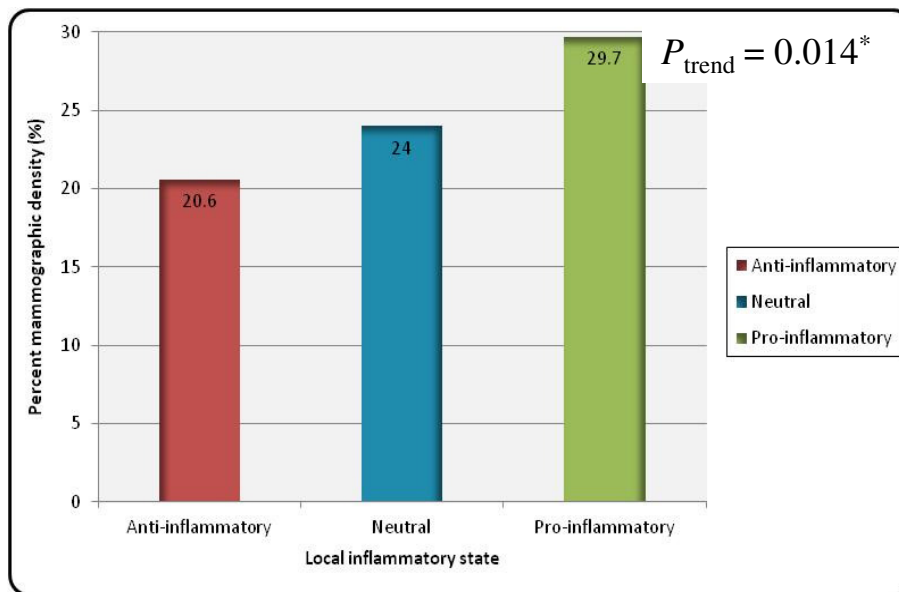
Further adjustment did not change the results

# Association between the expression of inflammatory markers and the percent mammographic density

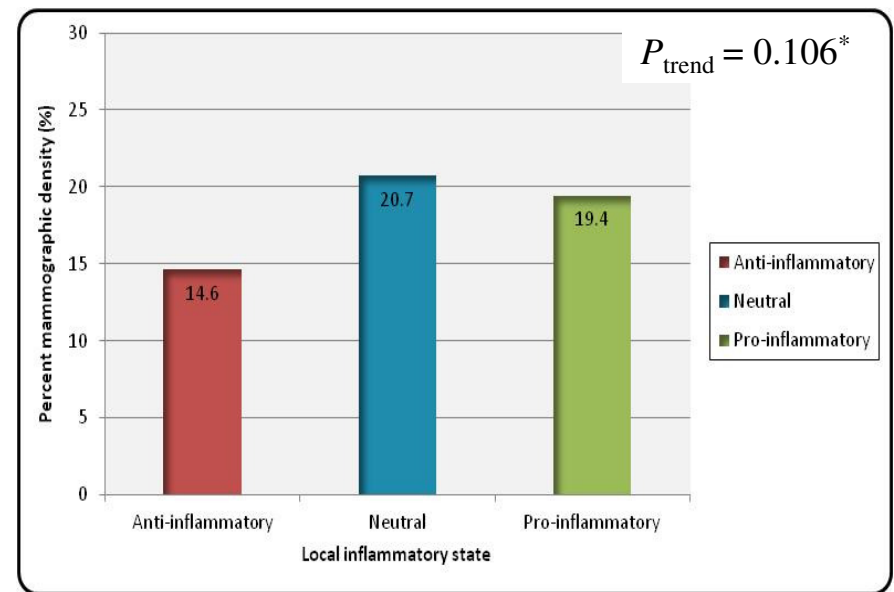
## TNF- $\alpha$ /TGF- $\beta$

### Stratified analyses by menopausal status

#### Premenopausal women



#### Postmenopausal women



\*Associations adjusted for age and waist circumference

Further adjustment did not change any of the results

# Conclusions and perspectives

- Pro-inflammatory state of IL-6/TGF- $\beta$  among all and postmenopausal women and TNF- $\alpha$ /TGF- $\beta$  among all and premenopausal women were associated with higher percent mammographic density compared to either the anti-inflammatory or the neutral state.
- Local inflammation in the breast tissue may induce cancer development through its effect on the mammographic density.

# Conclusions and perspectives

- Affecting the expression of inflammatory markers in breast tissue may provide attractive targets for future breast cancer preventive strategies.

# Aknowledgements

## Diorio Laboratory- Laval University

Dr Caroline Diorio (molecular epidemiologist)

Dr Bernard Têtu (pathologist)

Dr Simon Jacob (pathologist)

Isabelle Dumas (statistician)

Michèle Orain (research assistant)

Annick Michaud (laboratory assistant)



**CBCRA**  
Canadian Breast Cancer Research Alliance

**ACRCS**  
Alliance canadienne pour la recherche sur le cancer du sein

Thank you

