



ESCOLA SUPERIOR DE  
TECNOLOGIA DA SAÚDE  
DE LISBOA

**BioMedLab**  
ASSOCIAÇÃO PORTUGUESA DE CIÊNCIAS BIOMÉDICAS LABORATORIAIS

**H&TRC**  
Health and Technology Research Center

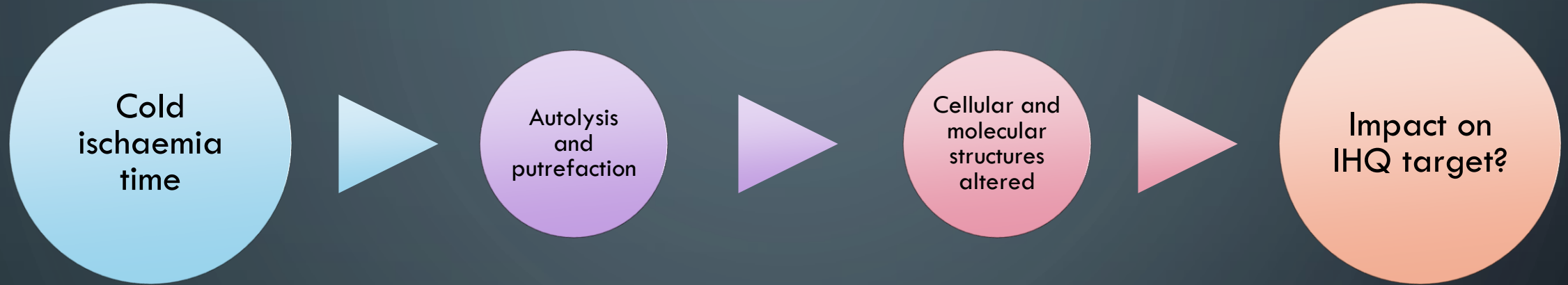
*THE IMPACT OF COLD ISCHAEMIA TIME ON THE  
IMMUNOLABELLING OF OESTROGEN RECEPTORS,  
PROGESTERONE RECEPTORS, AND HER2  
A SYSTEMATIC REVIEW*

MADALINA NOJE, ANA SILVA , MÁRIO MAIA-MATOS, AMADEU BORGES-FERRO

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**INTRODUCTION AND  
GOALS**

# METHODOLOGY



# MAIN GOAL

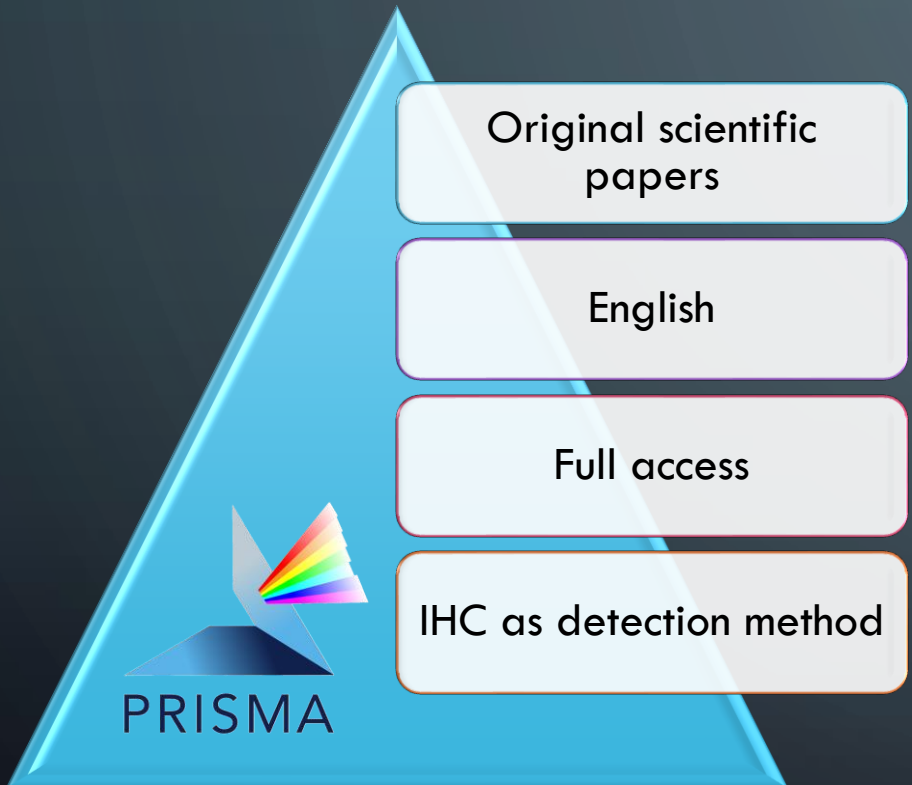
Evaluate the impact of cold ischemia time on the IHC labeling of ER, PGR and HER2.

Systematic Review

02

**METHODOLOGY**

# METHODOLOGY



## Data bases

- PubMed
- Web of Science



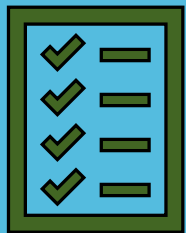
## MeSH terms

- *Cold ischemia time AND estrogen;*
- *Cold ischemia time AND progesterone;*
- *Cold ischemia time AND HER2.*

# METHODOLOGY

## **Checklist for Quasi-Experimental Appraisal Tool**

**Joanna Briggs Institute**



Are the goals of the study properly described?

Is the methodology properly described?

The dependent and independent variables are explicit?

Is there a control group?

Is the data collection process present and is it adequate?

Are the results presented accurately and in detail?

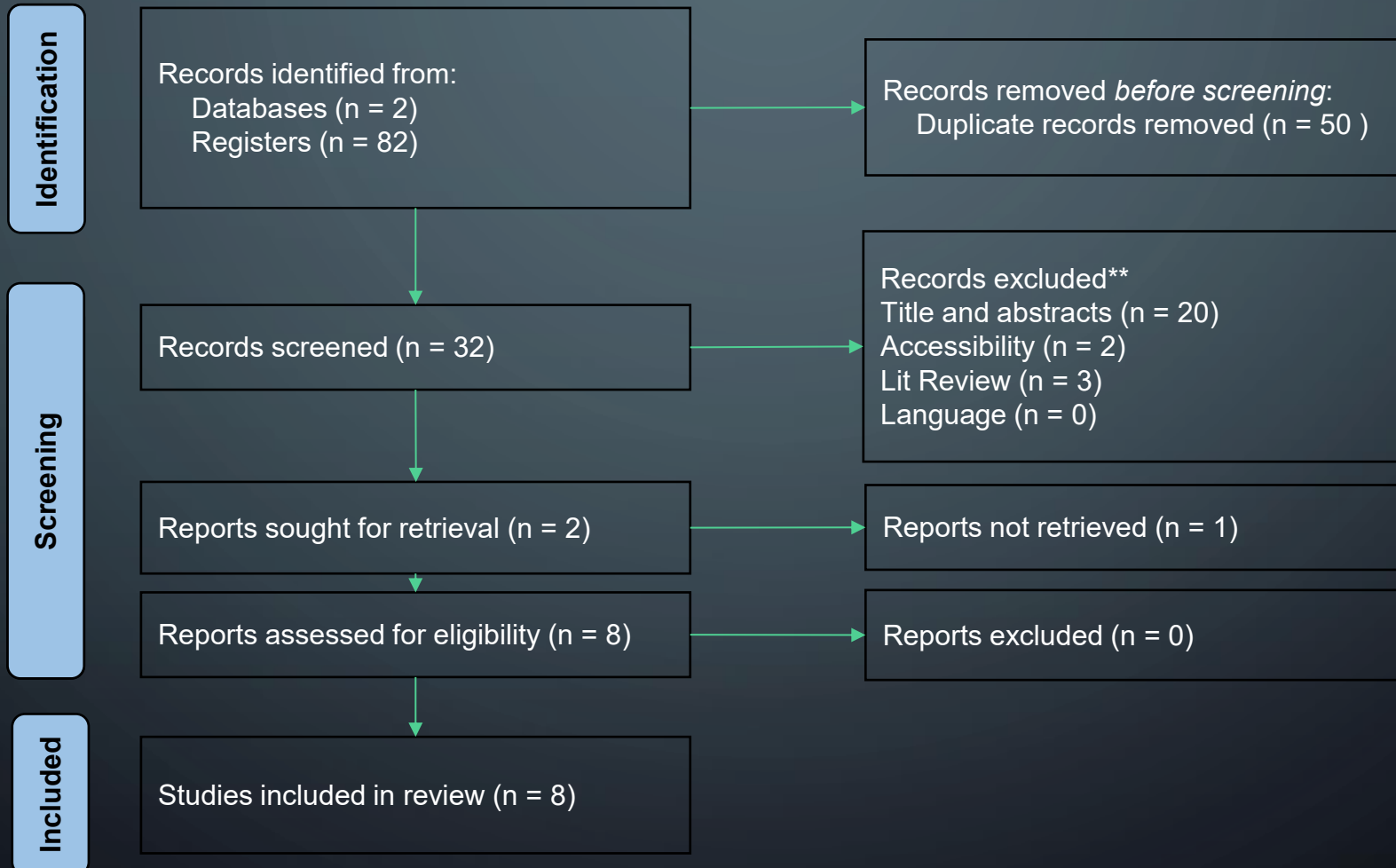
Are the conclusions supported by the results?

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**RESULTS**

# PRISMA FLOW DIAGRAM

## Identification of studies via databases and registers



# QUALITY ASSESSMENT

Autores \ Critérios	<i>Portier et al.</i>	<i>Khoury et al.</i>	<i>East et al.</i>	<i>Pekmezci et al.</i>	<i>Lee et al.</i>	<i>Li et al.</i>	<i>Yildiz-Aktas et al.</i>	<i>Qiu et al.</i>
Os objetivos do estudo são descritos de forma adequada?	Green	Green	Green	Green	Green	Green	Green	Green
É feita uma descrição adequada da metodologia?	Green	Green	Red	Red	Red	Green	Green	Green
As variáveis dependente e independente estão explícitas?	Green	Green	Green	Red	Green	Green	Green	Green
Existe um grupo controlo?	Green	Green	Green	Green	Green	Green	Green	Green
O processo de colheita de dados está presente e é adequado?	Red	Green	Green	Green	Red	Green	Green	Green
Os resultados são apresentados de forma precisa e detalhada?	Green	Green	Green	Green	Red	Green	Green	Green
As conclusões são sustentadas pelos resultados?	Green	Green	Green	Green	Green	Green	Green	Green

N = 6

# EXPERIMENTAL SETTINGS

	<i>Portier et al.</i> 2013	<i>Khoury et al.</i> 2009	<i>East et al.</i> 2019	<i>Li et al.</i> 2013	<i>Yildiz-Aktas et al.</i> 2012	<i>Qiu et al.</i> 2010
<b>Number of cases</b>	84	10 for each time	ER: 56 PGR: 56 HER2: 285	97	25 cold and 23 RT for each time	-----
<b>Cold ischemia time</b>	Between 27min and 6h25	0 min, 10 min, 30 min, 1h, 2h, 4h, 8h, overnight	≤4h Not controlled	Between 1 and 6 h	30 min, 1h, 2h, 3h, 4h, 24h, 48h	0 min, 10 min, 30 min, 1h, 2h, 4h, 8h, overnight
<b>Temperature</b>	-----	RT e 4°C	-----	-----	RT and cold	RT and 4°C
<b>Clones</b>	4B5 (HER2)	1D5 (ER), PgR636 (PGR) DAKO (HER2)	SP1 (ER), 1E2 (PGR), 4B5 (HER2)	6F11 (ER)	SP1 (RE), 1E2 (RP), 4B5 (HER2)	6F11, SP1 e 1D5 (ER); PgR636, 1E2 e 16 (PGR)

h- hours; min- minutes; ER- Oestrogen receptor; PGR- Progesterone receptor; RT- Room temperature

# OESTROGEN RECEPTOR

	<i>Khoury et al.</i>	<i>East et al.</i>	<i>Li et al.</i>	<i>Yildiz-Aktas et al.</i>	<i>Qiu et al.</i>
<b>Conclusions</b>	> 2h CIT < médium Q-score	5/56 ER category changed	5/97 H-score changed	RT - 11/23 (48%) 4°C - 8/25 (32%) reduced staining	< médium Q-score in all clones
<b>Statistic relevance</b>	Results are not statistically significant	-----	-----	RT - Stat sig >3h 4°C - Not stat sig	Statistically significant between 1D5 and SP1 @8h
<b>Study design</b>	CIT: 0 to ON Clone: PgR636	CIT: < 4h Clone: SP1	CIT: 1 to 6h Clone: 6F11	CIT: 0,5 to 48h Clone: SP1	CIT: 0 to ON Clone: 1D5, 6F11, SP1

# PROGESTERONE RECEPTOR

	<i>Khoury et al.</i>	<i>East et al.</i>	<i>Yildiz-Aktas et al.</i>	<i>Qiu et al.</i>
<b>Conclusions</b>	> 1h CIT < médium Q-score	5/56 PGR category changed	RT - 10/23 (43%) 4°C - 6/25 (24%) reduced staining	< médium Q-score in all clones
			RT > 4h neg 4°C > 24h neg	
<b>Statistic relevance</b>	Results are not statistically significant	-----	RT - Stat sig >24h 4°C - Not stat sig	Results are not statistically significant
<b>Study design</b>	CIT: 0 to ON Clone: 1D5	CIT: < 4h Clone: 1E2	CIT: 0,5 to 48h Clone: 1E2	CIT: 0 to ON Clone: PgR636, 1E2 e 16

# HER2

	<i>Portier et al.</i>	<i>Khoury et al.</i>	<i>East et al.</i>	<i>Yildiz-Aktas et al.</i>
<b>Conclusions</b>	Not affected	1 case 3+ not affected	38/285 category changed	RT - 11/23 (48%) 4°C - 6/25 (24%) reduced staining
<b>Statistic relevance</b>	-----	Results are not statistically significant	Statistically significant	
<b>Study design</b>	CIT: 0,5 to 6,5h Clone: 4B5	CIT: 0 to ON Clone: DAKO	CIT: < 4h Clone: SP1	CIT: 0,5 to 48h Clone: SP1

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**DISCUSSION**

# IS TIME DEPENDENT?

<1h

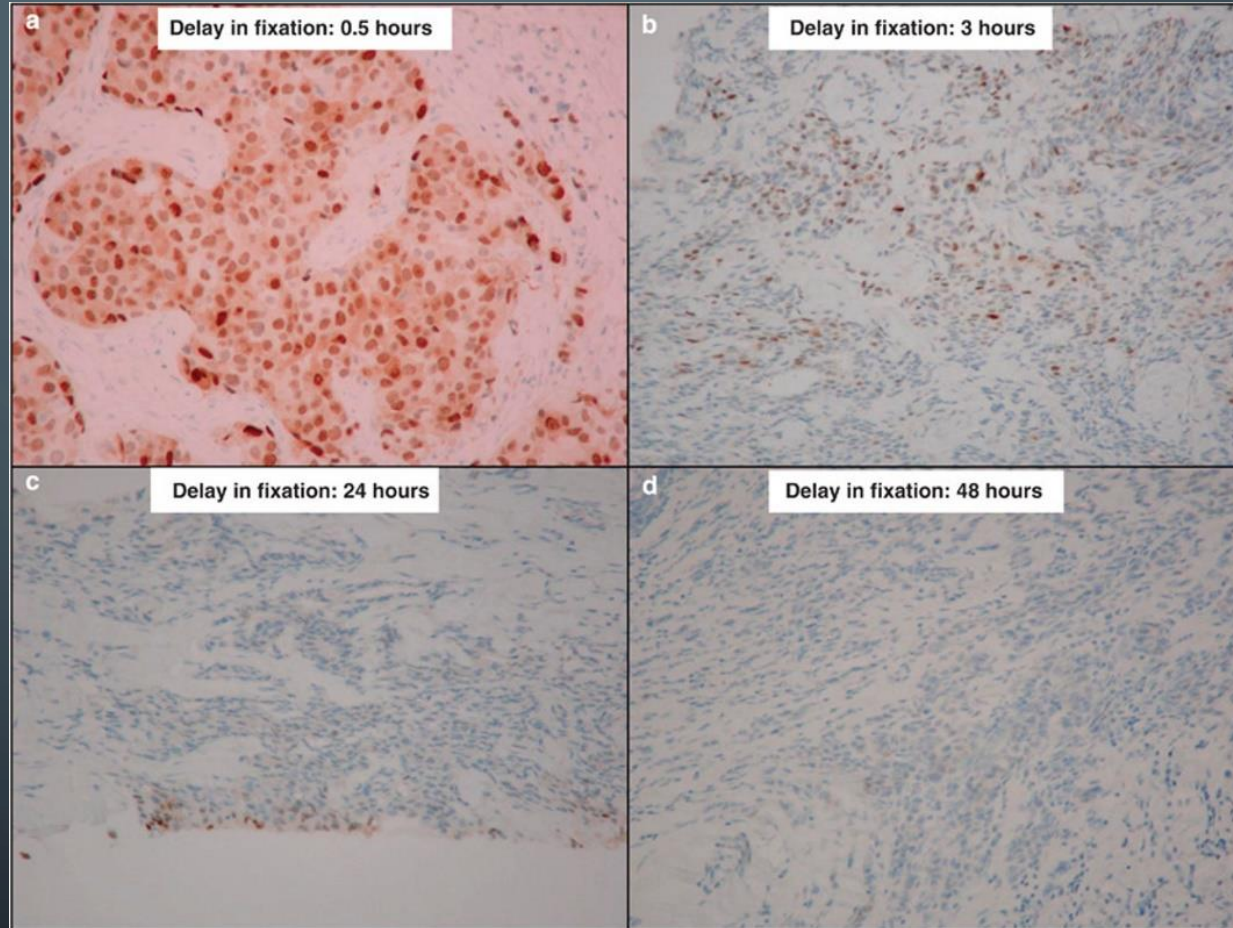
No major changes in  
biomarker status

1-8h

A tendency to decrease  
immunostaining

>24h

Most prominent impact on  
immunostaining

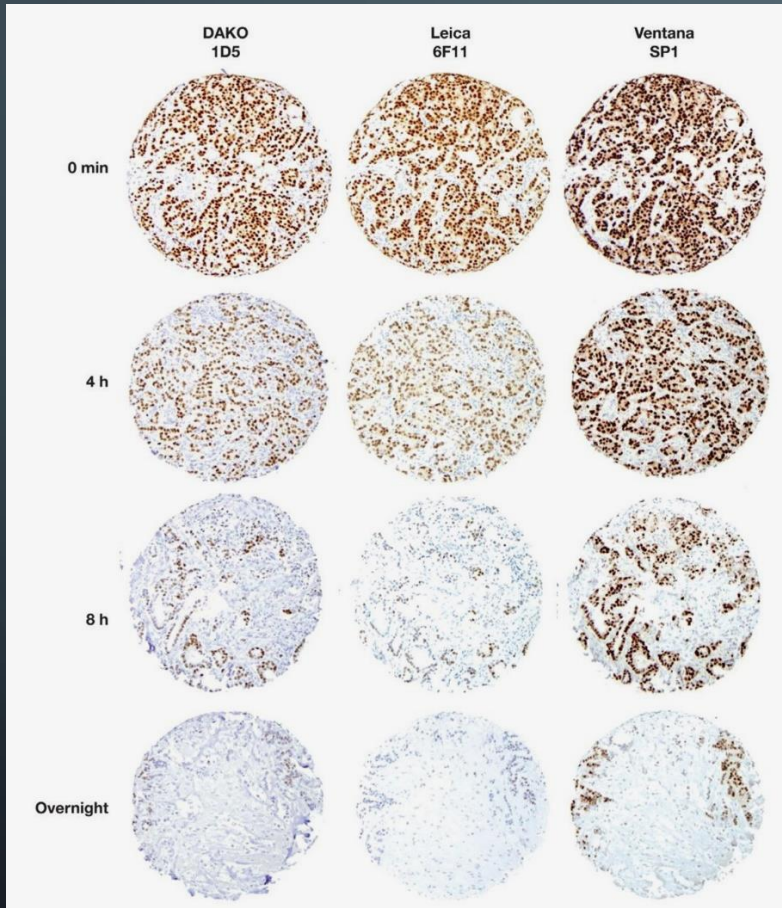


Progesterone  
Receptor

*Yildiz-Aktas IZ, Dabbs DJ, Bhargava R. The effect of cold ischemic time on the immunohistochemical evaluation of estrogen receptor, progesterone receptor, and HER2 expression in invasive breast carcinoma*

# IS CLONE DEPENDENT?

## Oestrogen Receptor



## Oestrogen receptors

More pronounced decrease in clones 1D5 and 6F11

## Progesterone receptors

Clone 1E2 showed different results in the different studies

## HER2

Clone 4B5: in two studies immunostaining decreased after 1h and in another study after 3h

# IS TEMPERATURE DEPENDENT?

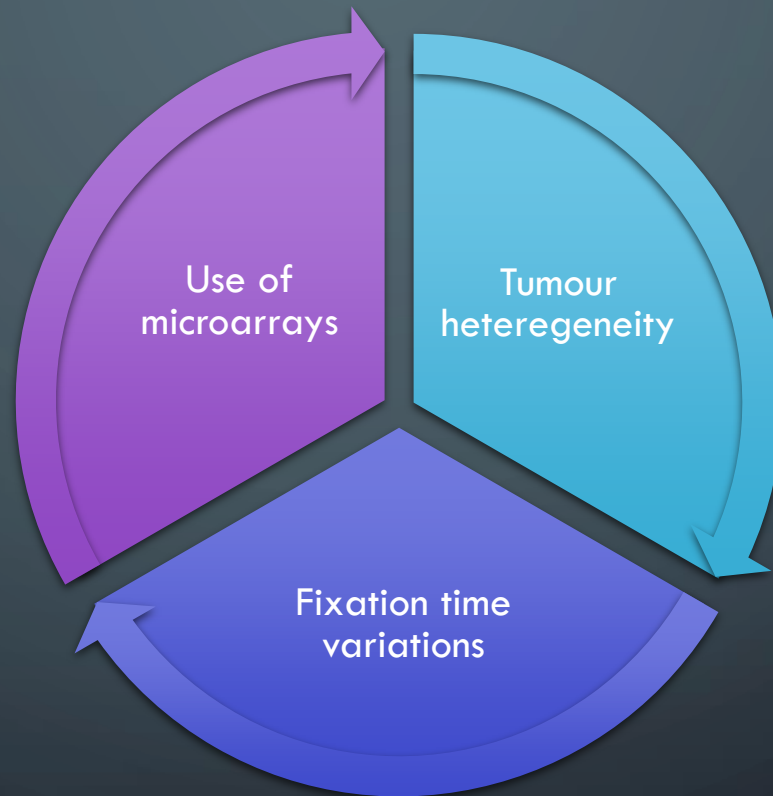
RT

Earlier decrease in immunostaining

Refrigerated

Decrease in immunostaining around 2 hours later compared to RT cases


# THERE ARE OTHER FACTORS?




05

**CONCLUSION**


# CONCLUSION




Progesterone receptors are more susceptible to degradation



60 minutes of cold ischaemia time is an acceptable period.  
30 minutes?



A decreasing trend in the immunostaining of receptors, have a direct impact at the clinical level



Low number of experimental articles on the subject

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