



# **Micro-RNAs as Biomarkers for Damage**

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- **Successful treatment of disease depends on early detection and appropriate therapy**
- **The presence of certain disease states can be identified by monitoring the expression levels of **biomarkers** (DNA, RNA, proteins)**
- **Biomarkers are an extremely important tool in areas like oncology, virology inflammation and heart disease**

## **Keren Zloto (2016)**

**Cardiac miRNAs as biomarkers for myocardial damage following heart surgery in children**

## **Or Bercovich (2017)**

**Immunomodulatory miRNAs following heart surgery in children**

## **Liat Mor (2017)**

**MiRNAs as biomarkers for brain damage following cardiac surgery in children**

## **Rachel Frenklak (2018)**

**Cardiac miRNAs as biomarkers for myocardial damage following TAVI**

## **Ortal Mentel (2019)**

**MiRNAs as biomarkers for brain anomalies in the fetus**

# Objective

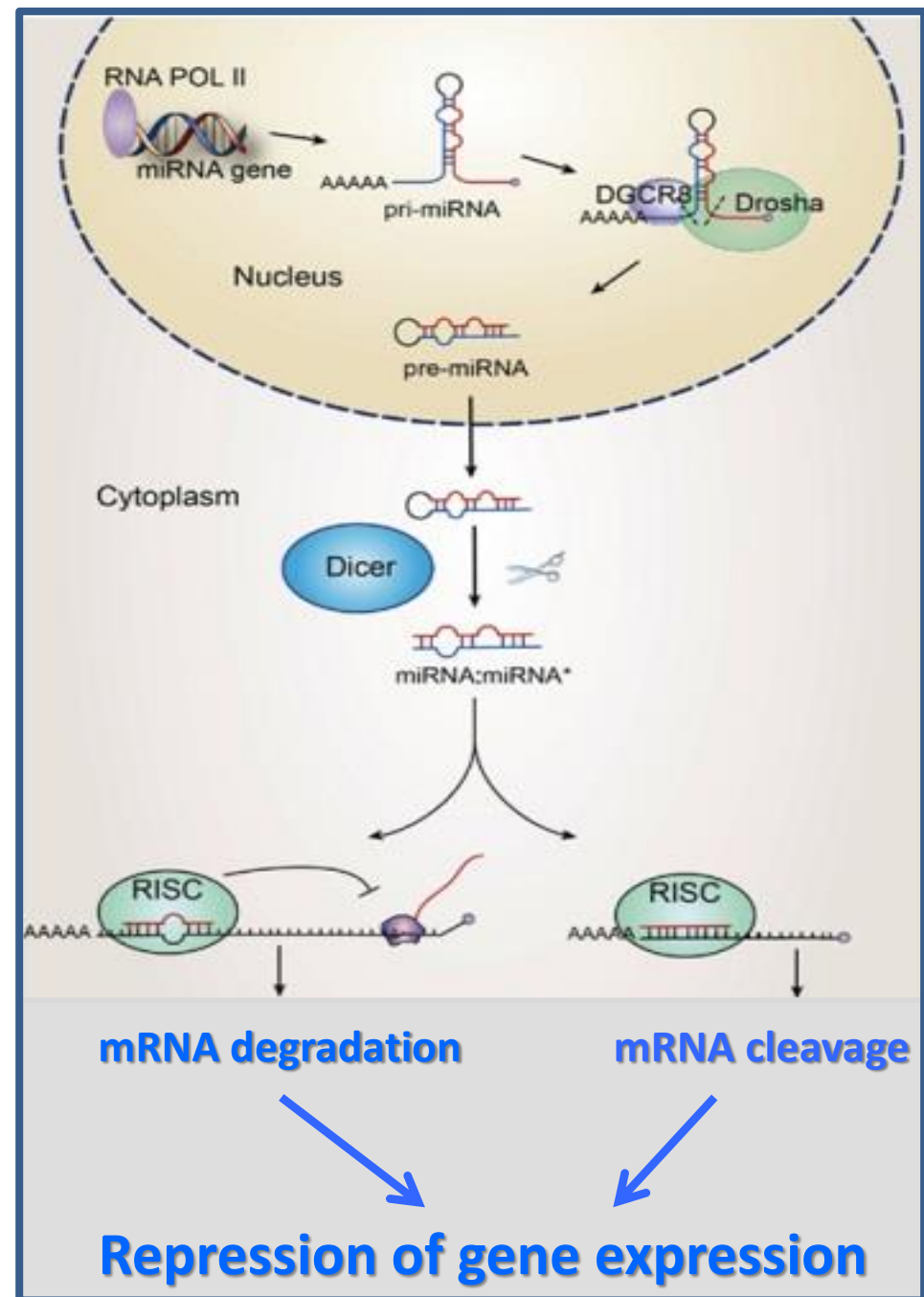
**Development of a diagnostic tool that will improve medical management and outcome of the patients**

**Adequate Biomarker should be:**

- **Stable**
- **Rapid release kinetics**
- **Specific to the organ we would like to monitor**
- **Detectable in a small sample of serum**

# Micro-RNAs (miRNAs)

- Short non-coding RNAs (~22 nt)
- Encoded by the DNA
- Transcribed by RNA Pol II
- Processed in the nucleus
- Exported to the cytoplasm
- Processed by Dicer
- Repress gene expression by:
  - mRNA degradation
  - mRNA cleavage
- Probably play a role in cell communication



# miRNAs

- Involved in all biological processes
- Tissue-specific expression pattern
- High biostability when excreted into body fluids (plasma, urine)

**Emerged as plasma biomarkers for many pathological states  
(cancer, diabetes, viral infections)**

## **Deciding on a new study**

- **Choose the medical situation for which there is need for a biomarker**
- **Choose the miRNA(s) to be checked (review of the literature)**
- **Define the patient groups for the study**
- **Helsinki**
- **Collect samples**

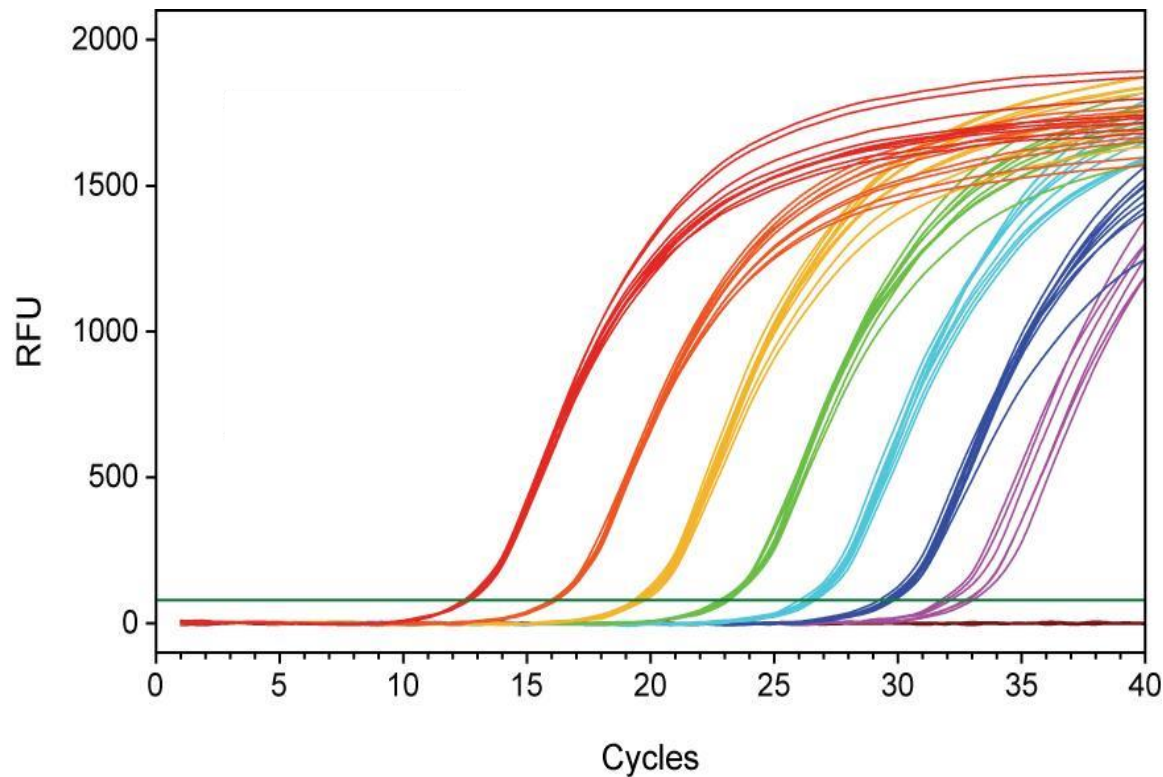
## How do we detect and quantify miRNAs?

- Extract total RNA from the sample of interest
- Prepare cDNA of the miRNA of interest
- Quantify by qPCR the miRNA using primers and a fluorescent probe
- Compare the amount of miRNA between different samples using a reference miRNA



# Quantative PCR (qPCR)

Amplification using specific primers and a fluorescent probe



- Following the molecular analysis...
- Collect the medical and demographic data of the participants
- Perform the relevant statistics

**Does the amount of the miRNA correlate with the “situation”?**

# **Micro-RNAs as Biomarkers for Myocardial Damage after Cardiac Surgery in Children**

- Incidence of congenital heart disease: 8/1000 births
- 50% of children with CHD (Congenital Heart Defect) will be operated for the repair of the defect during their first years of life
- Post-operative myocardial complications are a major cause for morbidity and mortality

**Can we predict which of the children will suffer  
from post-operative complications?**

# The Need

**Serum biomarkers for early and accurate detection of heart damage following pediatric cardiac surgery**

**The present biomarkers for detecting heart failure are insufficient as they suffer from lack of specificity (CPK, Troponin- the gold standard)**

**Additional biomarkers with increased predictive performances are needed for more precise and earlier prediction of complications after pediatric cardiac surgery**

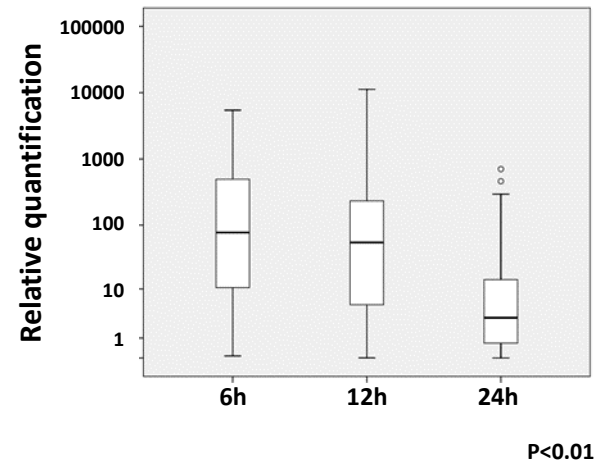
# Our Study

- **Samples were obtained from 79 pediatric patients with CHD, pre-operatively, 6h, 12h and 24h after the operation**
- **miRNAs were extracted from serum samples**
- **The relative amount of three miRNAs in plasma was measured by Quantitative RT-PCR (miR-208a, -208b, -499)**

# Levels of serum-derived miRNA-208a, -208b, -499 are highly elevated following cardiac surgery (samples before the operation=1)

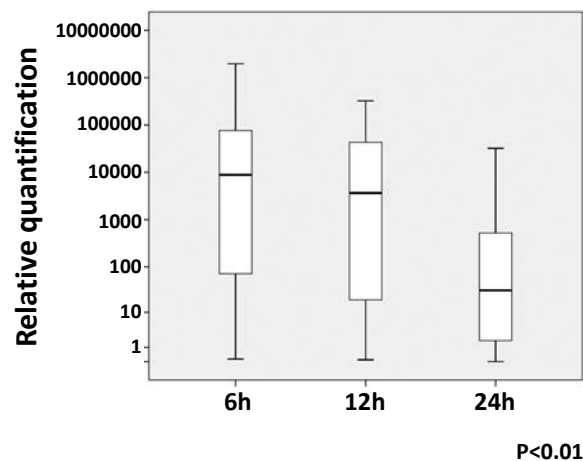
A.

miRNA-208a



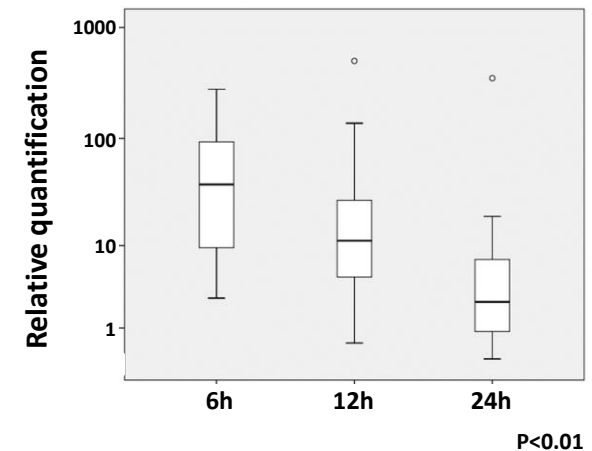
B.

miRNA-208b



C.

miRNA-499



## The amount of these miRNAs correlates with surgical parameters

		CPB time (min)	ACC time (min)
logRQ miR208a-6h	Corr. Coefficient Sig. (2-tailed)	.437** .001	.546** .000
logRQ miR208a-12h	Corr. Coefficient Sig. (2-tailed)	.255* .031	.362** .002
logRQ miR208a-24h	Corr. Coefficient Sig. (2-tailed)	.212 .092	.311* .012
logRQ miR208b-6h	Corr. Coefficient Sig. (2-tailed)	.294* .033	.428** .001
logRQ miR208b-12h	Corr. Coefficient Sig. (2-tailed)	.029 .809	.136 .256
logRQ miR208b-24h	Corr. Coefficient Sig. (2-tailed)	.161 .203	.226 .073
logRQ miR499-6h	Corr. Coefficient Sig. (2-tailed)	.473** .000	.434** .001
logRQ miR499-12h	Corr. Coefficient Sig. (2-tailed)	.376** .001	.448** .000
logRQ miR499-24h	Corr. Coefficient Sig. (2-tailed)	.194 .125	.263* .036

CPB – CardioPulmonary Bypass

ACC – Aortic Cross Clamp

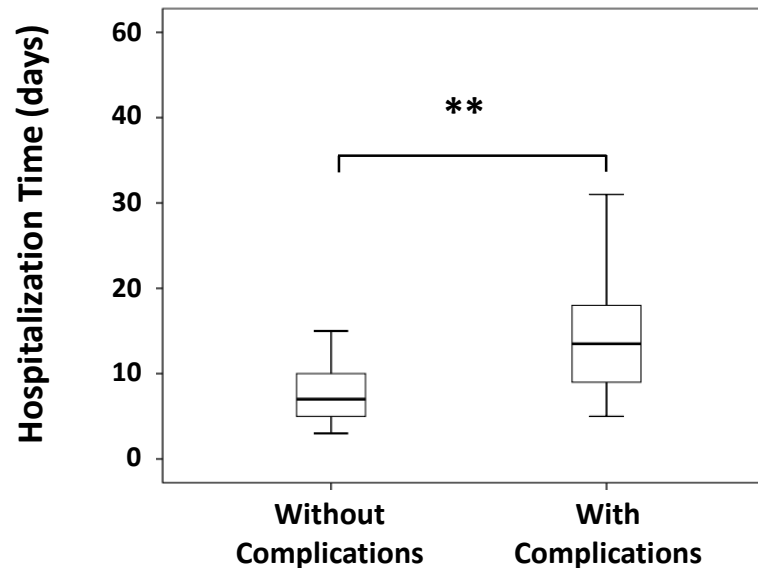
# The expression of miRNA-208a, -208b, -499 following cardiac surgery correlates with the laboratory parameters currently used to assess the patient's postoperative course



		Lactate	Troponin	Creatinine	ALT	AST
logRQ miR208a-6h	Corr. Coefficient Sig. (2-tailed)	.464** .000	.481** .000	.322* .020	.287* .037	.414** .002
logRQ miR208a-12h	Corr. Coefficient Sig. (2-tailed)	.299* .011	.427** .000	.214 .073	-.031 .796	.290* .013
logRQ miR208a-24h	Corr. Coefficient Sig. (2-tailed)	.121 .341	.365** .003	.085 .508	-.069 .586	.254* .043
logRQ miR208b-6h	Corr. Coefficient Sig. (2-tailed)	.425** .002	.432** .001	.289* .038	.146 .297	.205 .140
logRQ miR208b-12h	Corr. Coefficient Sig. (2-tailed)	.272* .021	.219 .065	.119 .322	-.181 .128	.007 .953
logRQ miR208b-24h	Corr. Coefficient Sig. (2-tailed)	.152 .229	.479** .000	-.046 .718	.037 .774	.376** .002
logRQ miR499-6h	Corr. Coefficient Sig. (2-tailed)	.318* .020	.598** .000	.160 .259	.349* .010	.479** .000
logRQ miR499-12h	Corr. Coefficient Sig. (2-tailed)	.213 .073	.613** .000	.111 .356	.036 .765	.480** .000
logRQ miR499-24h	Corr. Coefficient Sig. (2-tailed)	.000 .999	.523** .000	-.054 .672	.047 .713	.446** .000



# Correlation of Hospitalization time with Complications



The amount of each of the 3 miRNAs at 12 h correlated with the length of hospital stay ( $p < 0.05$ )

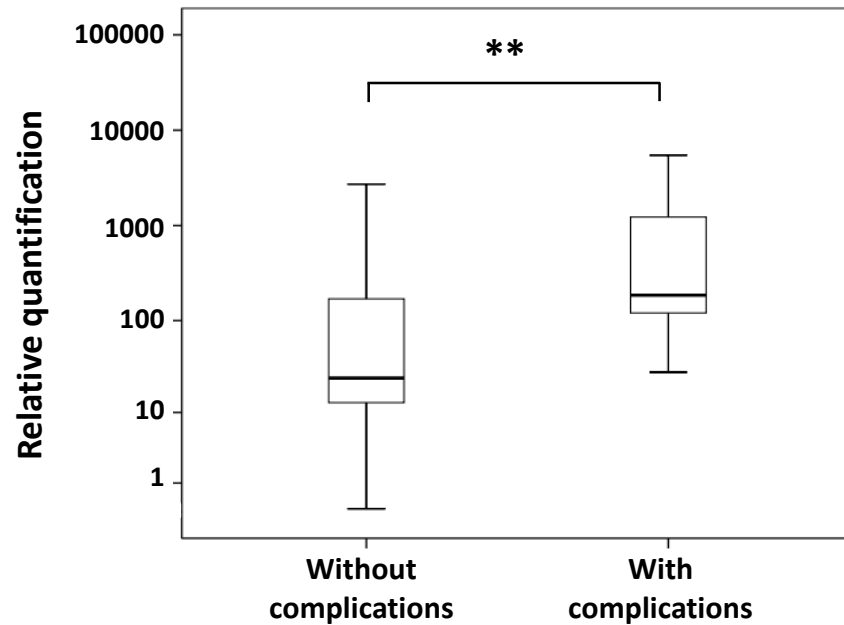
- No association between surgical parameters (CPB, ACC) or troponin levels to the occurrence of complications

The complexity of surgery is not predictive of a complicated postoperative course

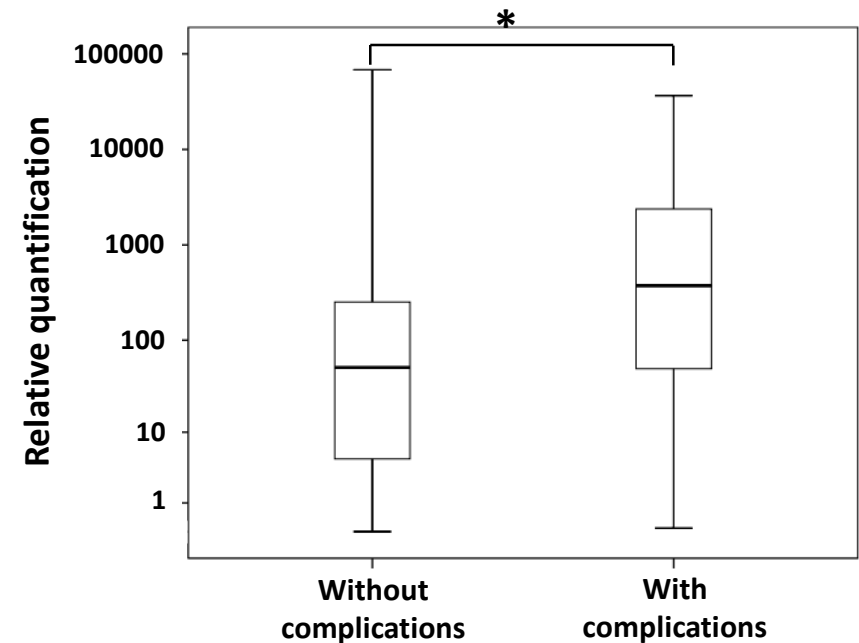
However...

# miRNA-208a correlates with the appearance of complications

miRNA-208a 6h

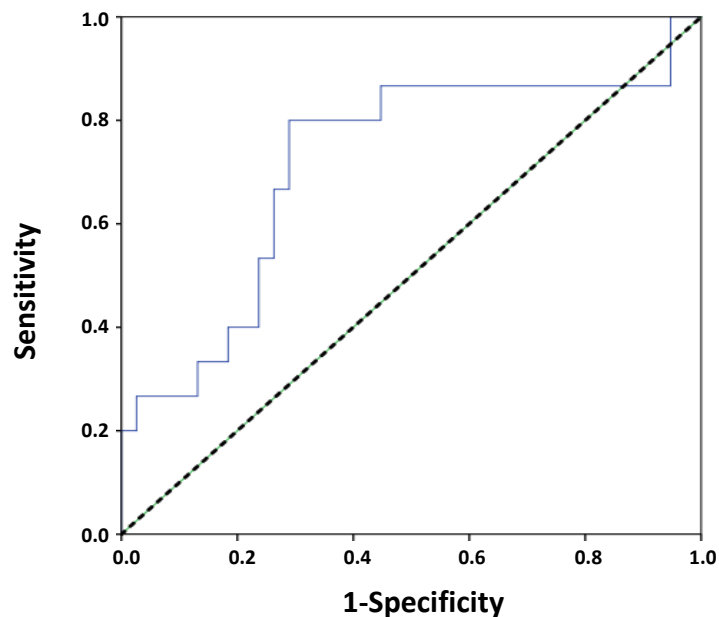


miRNA-208a 12h



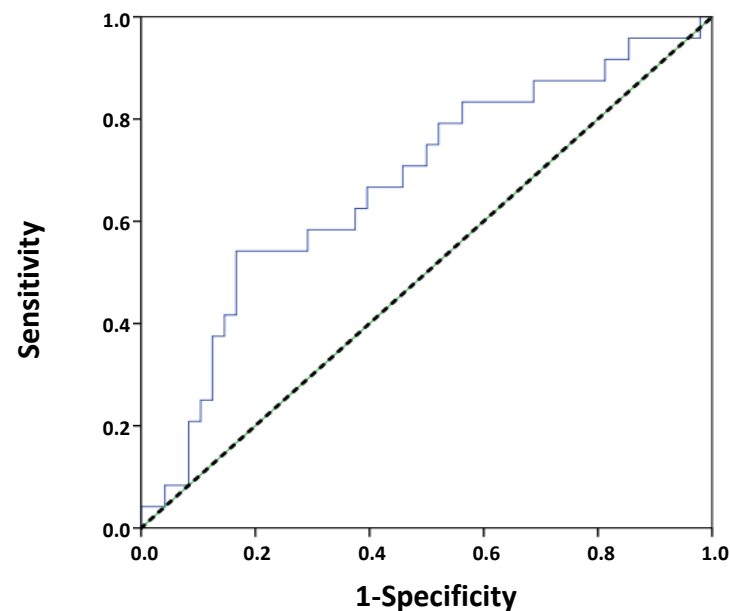
## The ability of miRNA-208a to predict complications

6 h



**AUC of 71.6%**  
**(95%CI: 54.9–88.2%;  $p = 0.01$ )**

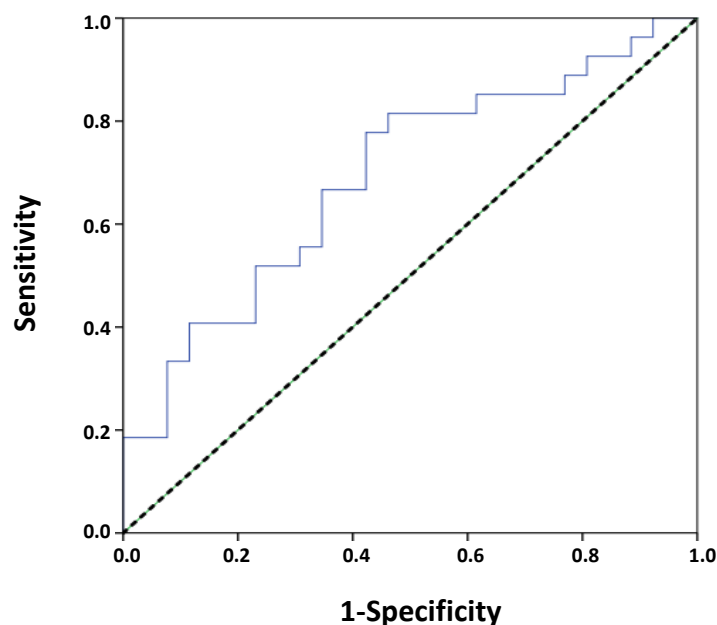
12 h



**AUC of 67.3%**  
**(95%CI: 53.8–80.8%;  $p = 0.01$ )**

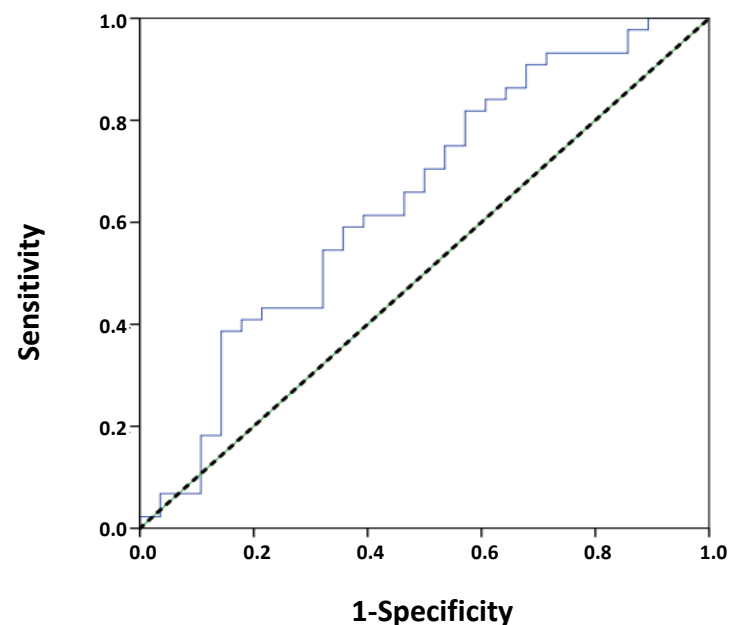
# The ability of miRNA-208a to predict the possibility of being ventilated more than 48 h following surgery

6 h



**AUC of 69.2%**  
(95%CI: 55%-83.5%,  $p = 0.01$ )

12 h



**AUC of 64.4%**  
(95%CI: 51-77.9%;  $p = 0.04$ )

## Summary

- **MiRNAs-208a, -208b, -499 can be detected in the blood of pediatric patients undergoing heart surgery**
- **The relative amount of the 3 miRNAs is in correlation with the surgical and laboratory parameters**
- **The amounts of these miRNAs in the serum of patients following surgery correlate with myocardial damage**

## Conclusions

**Circulating miRNA-208a in serum is a sensitive and specific predictor for the risk of developing complications during the postoperative course as early as 6 h after heart surgery in pediatric patients with CHD**

## Ortal's project

### MiRNAs as biomarkers for brain anomalies in the fetus

Joint project with Dr. Eldad Katorza

- Early detection of brain malformations in the fetus is of great importance, influencing decisions made about pregnancy management and delivery
- To date, prenatal diagnosis of such defects is performed mainly by ultrasonography and MRI, sometimes only during the third trimester of pregnancy although the defects are present at much earlier stages.

**Can we find a biomarker for brain malformations which will give an early and precise indication of such events in the fetus?**



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המחלקה לטיפול נמרץ ילדים  
בית החולים אדמונד ולילי ספרא לילדים



**Thank-you!**

**Good luck!!!**



## The type of operation that each of the 79 patients underwent

<u>Type of operation</u>	<u>Number of patients</u>	<u>% of patients</u>
VSD/ASD	17	22
AV CANAL	4	5
TGA	13	17
TOF	9	11
COA+arch repair	7	9
BT shunt	5	6
Norwood	5	6
Glenn	5	6
Fontan	6	8
RV to PA conduit	8	10
Total	79	100

## **Surgical characteristics of the operated children**

<b>Days of Hospital Median</b>	<b>8 (6-13)</b>
<b>Days ventilated non-invasive Median</b>	<b>1.5 (0.33-4.20)</b>
<b>Days ventilated invasive Median</b>	<b>0.87 (0.21-1.97)</b>
<b>Reintubation/ no Reintubation</b>	<b>16/59</b>
<b>ECMO / no ECMO</b>	<b>0/75</b>
<b>Max. Inotropic score Median</b>	<b>14.9 (5-22.5)</b>
<b>Inotropic support days Median</b>	<b>2 (1-3.5)</b>
<b>CPB time (min.) Median</b>	<b>62 (30-100)</b>
<b>Aortic cross clamp (min.) Median</b>	<b>30 (0-70)</b>
<b>Respiration before op. (days) Median</b>	<b>0 (0-0.75)</b>
<b>Complications/Mortality/ No complications</b>	<b>22/4/53</b>