Evaluation of miR-146a and miR-155 plasma expression levels in patients with Mycosis Fungoides and detection of single nucleotide polymorphisms in their sequence

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INTRODUCTION

Diagnosis of Mycosis Fungoides (MF) is often delayed, especially at the early stages, because of its polymorphic nature and its resemblance to benign inflammatory disorders.

MicroRNAs are small non-coding RNA molecules, that regulate genes' expression through epigenetic modulation. MiRNAs exhibit high stability in biological fluids, resisting in degradation and in detrimental conditions such as temperature and pH variations. Thus, a plethora of extracellular miRNAs have been suggested as putative biomarkers in many malignancies including Cutaneous T-Cell Lymphomas.

Objective: The aim of the present study was to analyze the expression levels of microRNAs (miR) -146a and -155 in the plasma of MF patients and healthy volunteers and to detect the presence of Single Nucleotide Polymorphisms (SNPs) in their genes.

METHODS

The appropriate sample size for statistical power equal to 0.94 **(1-β=94%)** and significance level at 0.05 **(α=5%)** was determined **by a pilot study**, using G.Power 3.1.9.2. The optimal sample size was calculated at **n=82**, with a ratio of **MF patients/healthy individuals equal to 1:1**.

MiRs' expression was evaluated with **qRT-PCR**, and the relative quantity was calculated by the **$2^{-\Delta Ct}$ method** using **cel-mir-39** as reference gene.

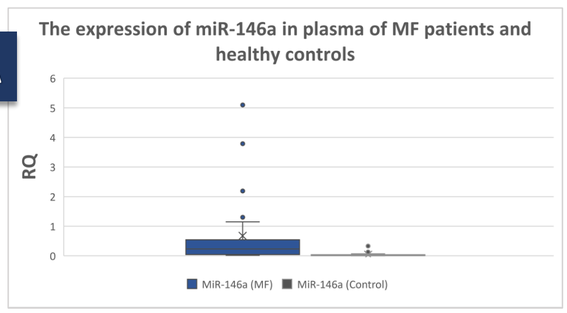
In a subset of patients, the promoter region and/or the pre-miRNA genomic region of these miRs were scanned for the presence of SNPs in DNA extracted from white blood cells, **by Sanger sequencing**.

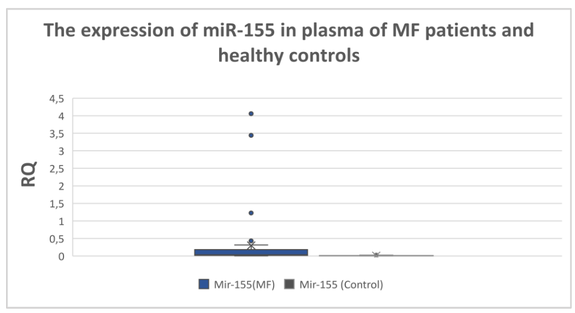
Statistical analysis was performed with the statistical package **IBM SPSS 25**

MF patients	n (%)
Total Number	41
Median age (range)	59 years (31-85)
Females	11 (26.83%)
Males	30 (73.17%)
Early Stage	
IA	22 (53.65%)
IB	8 (19.51%)
IIA	2 (4.87%)
Advanced Stage	
IIB	5 (12.19%)
III	3 (7.31%)
IV	1 (2.47%)

RESULTS

A





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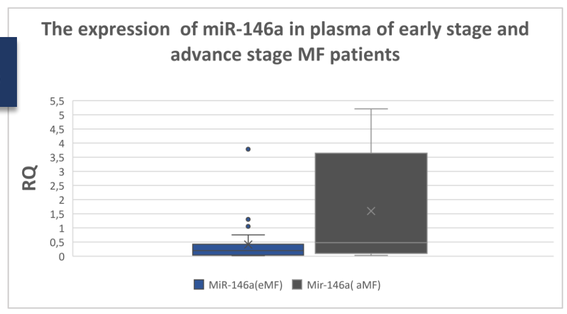

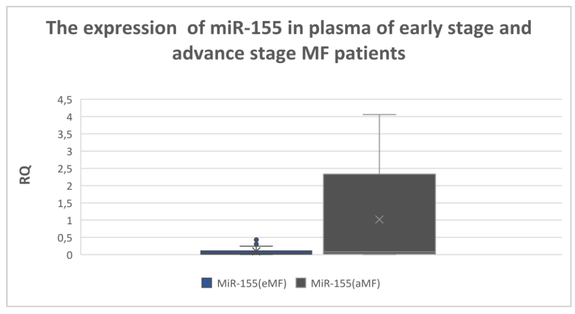

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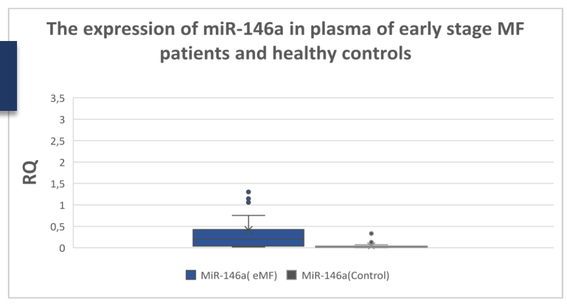
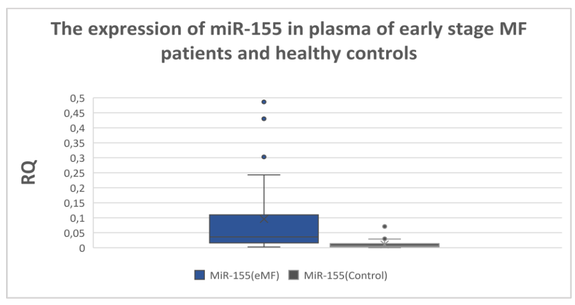
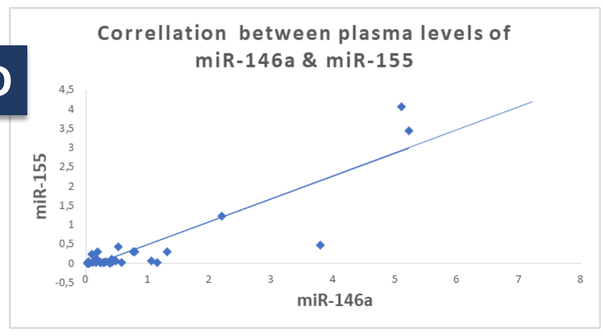




	miR-146a			miR-155		
	Mean	**SD**	**p-value**	**Mean**	**SD**	**p-value**
MF patients vs controls	0.674212	1.23564	**0.001**	0.29918	0.82278	**0.028**
eMF patients vs controls	0.035288	0.06244	**0.001**	0.01112	0.01362	**<0.001**
eMF vs aMF patients	0.41457	0.70222	**0.009**	0.09590	0.13090	**0.002**
gender correlation	0.42245	0.37574	0.437	0.095385	0.14064	0.343
age correlation			0.079			0.311
correlation between plasma levels of two miRs			**<0.001**			**<0.001**

D



miR-146a rs2910164 (C>G) polymorphism		
	MF patients (n=20) MF alleles (2n=40)	**Controls (n=11) Controls' alleles (2n=22)**
Genotype Frequency (%)		
GG	10/20=0.5 (50%)	4/11=0.363 (36.3%)
GC	9/20=0.45 (45%)	6/11= 0.545 (54.5%)
CC	1/20=0.05 (5%)	1/11=0.092 (9.2%)
Allele's frequency		
Allele G	29/40=0.725 (72.5%)	14/22= 0.62 (62%)
Allele C	11/40=0.275 (27.5%)	8/22= 0.38 (38%)

miR-155 rs767649 (T>A) polymorphism		
	MF patients (n=17) MF alleles (2n=34)	**Controls (n=10) Controls' alleles (2n=20)**
Genotype Frequency (%)		
TT	13/17=0.76 (76%)	9/10=0.9 (90%)
TA	3/17=0.18 (18%)	1/10= 0.1 (10%)
AA	1/17=0.06 (6%)	0/10=0
Allele's frequency		
Allele T	29/34= 0.85 (85%)	19/20= 0.96 (96%)
Allele A	5/34= 0.15 (15%)	1/20= 0.04 (4%)

CONCLUDING REMARKS

- No SNPs were detected in the pre-miR-155 sequence of MF patients after alignment with the reference sequence through Emboss Needle. There was no significant difference in the genotype patterns between patients and controls for either rs2910164 nor rs767649. The presence of SNPs regulating mirs' function may need to be evaluated in larger cohorts.
- Plasma levels of miR-146a and miR-155 were significantly higher in MF patients vs controls **(A)**, in early vs advanced stages **(B)** and in early MF patients vs controls **(C)**. A positive correlation was detected between plasma levels of miR-146a and miR-155 in patients' samples **(D)**.
- The detection of increased mir-146a and mir-155 plasma levels in MF patients is a promising finding in the attempt to establish putative non-invasive biomarkers for prompt diagnosis, prognosis, and adequate therapy of these patients.

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