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MicroRNAs immune response profile to COVID-19 vaccine boost in cancer patients under active treatment

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Abstract

Cancer patients are at increased risk of severe COVID-19 outcomes. Understanding their immunological response to SARS-CoV-2 mRNA vaccination is essential for identifying molecular biomarkers that can predict vaccine efficacy. MiRNAs are promising biomarkers because they regulate immunity-related genes through virus-host interactions. This study aims to validate a specific miRNA profile in cancer patients to assess their immune response to COVID-19 vaccination and the influence of these miRNAs on humoral, cellular immunity and systemic inflammation. This study includes three cohorts: Cohort 1 (56 cancer patients with haematological and solid tumours) with blood samples collected before, -3 and − 6 months post-boost, Cohort 2 (209 cancer patients) and Cohort 3 (86 healthy individuals) with samples collected − 3 and − 6 months post-boost. Cellular immunity was evaluated in 104 cancer patients by measuring IFN-γ production post-booster vaccination. In solid cancer patients from Cohort 1, the expression of hsa-miR-7-5p, hsa-miR-15b-5p, and hsa-miR-98-5p increased three months after the booster dose and, in haematological patients, a similar trend was observed for hsa-miR-98-5p. When analysing all patients from cohorts 1 and 2, at -3 and − 6 months post-booster, a decrease in hsa-miR-7-5p was observed in solid cancer patients, while both hsa-miR-98-5p and hsa-miR-7-5p levels declined in haematological patients 6 months post-boost, possibly due to the declining of vaccine-induced inflammation. A significant positive Spearman correlation was found between hsa-miR-7-5p expression and IgG S levels. Additionally, IFN-γ non-reactive patients showed higher hsa-miR-7-5p levels compared to IFN-γ positive patients, suggesting a potential influence on both humoral and cellular immune response. Furthermore, patients with high expression of hsa-miR-24-3p exhibited significantly higher platelet-to-lymphocyte ratio (PLR) while high expression of hsa-miR-7-5p was significantly associated with elevated of both PLR and systemic immuno-inflammation index (SII) indices suggesting a potential correlation between these miRNAs and systemic inflammatory status. Accordingly, hsa-miR-7-5p may represent a key biomarker integrating immune and inflammatory regulation in cancer patients. Identifying potential biomarkers could enhance personalized medicine approaches, enabling more precise management of high-risk cancer patients while considering their influence on immune response development and systemic inflammation in the context of COVID-19 vaccination.

1. Introduction

Coronavirus disease (COVID-19) pandemic was caused by the highly contagious pathogen severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) that led to millions of cases of infection and deaths worldwide [1, 2]. In severe cases, SARS-CoV-2 infection can lead to pneumonia and inflammation causing acute respiratory distress syndrome (ARDS) which can promote hyperinflammatory state, lymphocytopenia, organ damage, and even death [3, 4]. Consequently, due to the huge impact on public global health, this pandemic has caused an urgent necessity for vaccine development promoting the emergence of mRNA vaccines against SARS-CoV-2, the main vaccines administered worldwide [5–7]. SARS-CoV-2 mRNA vaccination may induce an effective humoral and cellular immunity. However, since cancer patients were not included in the pivotal clinical trials, there is a significant lack of information relating to vaccine efficacy on triggering immune response and consequent adverse effects [8, 9]. To date, most studies have analyzed the antibody response elicited through vaccination, associating the reduced humoral response with individual risk factors such as advanced age, active malignancy, and treatment with some anticancer therapies, including B cell-depleting treatments, which put these patients at a higher risk of breakthrough infections [10–13]. Indeed, in a recent work of our research group, we evaluated the humoral immune response of cancer patients to COVID-19 vaccination through IgG S levels quantification and we verified that they had a weaker immune response to the SARS-CoV-2 vaccination compared to healthy individuals and, among cancer patients, those with haematological cancer had a worse response to

than those with solid cancers [14]. Moreover, other clinical variables such as chemotherapy and high febrile neutropenia risk significantly impaired the COVID-19 vaccination efficacy [14]. In parallel to the humoral immune response, it is crucial to gain a deep understanding of the cellular immune response of these patients to COVID-19 vaccination [14–16]. Evidence regarding the role of cellular immunity in COVID-19 is emerging, suggesting that this immunity may be maintained even in the absence of humoral response [17, 18]. Memory T cells significantly contribute to effective protection against viral exposure, highlighting the crucial role of cellular immunity in preventing severe disease [18]. Consequently, the study and analysis of both T-cell and humoral responses against SARS-CoV-2 are fundamental to better understanding the immune response to vaccination [18]. COVID-19 vaccines are expected to prompt an effective cellular immune response. Indeed, interferon-gamma (IFNγ) secretion by SARS-CoV-2-specific $CD4^+$ and $CD8^+$ T lymphocytes is associated with improved COVID-19 outcomes [19, 20]. IFN-γ release by T-cells has gained attention in the research field, as IFN-γ T-cell responses provide valuable insights into SARS-CoV-2 specific cellular immunity [18]. Therefore, assessing IFN-γ levels is a key method for studying SARS-CoV-2 T-cell memory [16, 20, 21]. Moreover, to better understanding of the immune response of cancer patients to COVID-19 vaccination is crucial to identify molecular biomarkers useful to stratify patients according to their immune response to vaccination. This stratification approach could be implemented in the clinical practice in the future, allowing the improvement of disease management in cancer patients. MicroRNAs (miRNAs) are considered highly promising biomarkers with the ability to regulate immune-related gene targets through virus-host cell interactions [22, 23]. Numerous miRNAs have been described as crucial factors in SARS-CoV-2 infection, regulating inflammation, performing antiviral functions, and in the modulation of the immune response [24, 25]. Furthermore, there are several studies demonstrating that miRNAs can inhibit SARS-CoV-2 replication, Spike expression, and are also able to regulate ACE2 levels [26–29]. In addition, it is already well established that miRNAs expression is deregulated in several human malignancies, with an impact on patients' prognosis and response to treatment [30–34]. Therefore, it is possible that cancer patients with an aberrant expression of miRNAs that are also able to regulate the expression of Spike protein will be more prone to vaccination failure. A previous review article from our research group proposed a miRNA profile of seven SARS-CoV-2-related miRNAs five that target Spike protein sequences (hsa-miR-7-5p, hsa-miR-24-3p, hsa-miR-145-5p, hsa-miR-223-3p and hsa-miR-15b-5p) and two other that target S2 subunit and improves the fusion of viral and host cell membranes that is activated by the transmembrane protease serine 2 (TMPRSS2) (hsa-miR-214 and hsa-miR-98-5p) [26, 35, 36]. Besides, several studies have demonstrated that miRNAs are crucial regulators of inflammatory responses, acting through the modulation of immune cell function, cytokine signalling and inflammation-related molecular pathways [37, 38]. Given that chronic inflammation is a well-established hallmark of cancer, contributing to tumour progression and immune escape, dysregulation of miRNA expression may play a critical role in shaping the inflammatory tumour microenvironment [37, 39]. In cancer patients, systemic inflammation has been consistently associated with disease progression and clinical outcomes. Systemic inflammatory indices derived from routine peripheral blood counts have gained increasing attention as accessible biomarkers of this inflammatory status including platelet-to-lymphocyte ratio (PLR), systemic immuno-inflammation index (SII), neutrophil-to-lymphocyte ratio (NLR) and lymphocyte-to-monocyte ratio (LMR) [40–43]. The PLR, calculated as the ratio of platelets to lymphocytes, is a widely used marker of systemic inflammation and platelet activation. Elevated PLR reflects systemic inflammation, predict infections and comorbidities and has been associated with immune dysregulation and with cardiovascular, inflammatory, and neoplastic diseases [44–46]. The NLR, a simple ratio of neutrophils to lymphocytes, reflects immune dysregulation and has been strongly associated with outcomes in infectious diseases, autoimmune disorders, cancer, and post-surgical recovery [41, 47]. SII which combines neutrophil, lymphocyte, and platelet counts, provides a more comprehensive measure of systemic inflammation. Subsequently, numerous researchers have linked SII to various diseases, including

tumours, infectious diseases, and cardiovascular diseases [41, 48]. High SII values have been linked to poorer outcomes in cancer, infectious, and cardiovascular diseases, supporting its use as a prognostic biomarker [49]. The LMR indicates the balance between immune competence and monocyte-driven inflammation. Reduced LMR has been linked to worse outcomes in cancer and inflammatory diseases, reinforcing its role as a marker of systemic immune and inflammatory status [43, 44]. Therefore, given the importance of systemic inflammation in these patients, it is important to evaluate whether miRNAs are associated with systemic inflammatory indices.

In the present study, we intend to validate this miRNA profile in cancer patients in the context of SARS-CoV-2 vaccination response and to explore how these miRNAs relate to the development of humoral and cellular immune responses and impact systemic inflammation.

2. Material and Methods

2.1 Ethics Approval and Consent to Participate

This project received approval from the Ethics Committee of the Portuguese Oncology Institute of Porto (IPO-Porto) (CES IPO: 286/021). All the patients enrolled in the study signed a written informed consent, under the principles of the Helsinki Declaration. The informed consent form is provided in the supplementary materials. Clinical trial number: not applicable.

2.2 Study Population

This study included patients over the age of 18, admitted at the IPO-Porto with hematologic or solid cancers and undergoing active treatment, who were eligible for a COVID-19 vaccine booster dose. The primary vaccines administered were mRNA-based vaccines, such as Pfizer and Moderna. The study included both hematologic and solid cancer patients who had received SARS-CoV-2 booster doses: Cohort 1 included 56 patients (12 with haematological and 44 with solid tumors) from whom samples were collected at three timepoints — Timepoint -1, Timepoint 1, and Timepoint 2; Cohort 2 included 209 patients (29 with haematological and 180 with solid tumors) with samples collected at Timepoint 1 and Timepoint 2; and Cohort 3 included 86 healthy individuals, also eligible for a booster dose of COVID-19 vaccination, with samples collected at Timepoint 1 and Timepoint 2. For Cohort 1, the first sample was taken before the booster dose (timepoint -1), and the subsequent samples were collected 3 (timepoint 1) and 6 months (timepoint 2) following the booster. In Cohorts 2 and 3, samples were collected 3 (timepoint 1) and 6-months (timepoint 2) post-booster. Demographic and clinical information of Cohorts 1 and 2 are presented in Table 1. Cohort 3, the healthy group (N = 86), consisted of 14 males (16.3%) and 72 females (83.7%), with mean ages of 54.9 and 53.5 years, respectively.

Table 1- Clinical characteristics of patients with cancer included in this study in either cohort 1 or cohort 2.

		Gender	
		Male, n (%)	**Female, n (%)**
Patients, n (%)	284 (100%)	140 (49.3%)	144 (50.7%)
Patient Age (mean ± SD)	61.79±11.16	63.67 ± 10.50	59.96 ± 11.48
SOLID TUMOR CASES, n (%)	**240 (84.5%)**	**118 (49.2%)**	**122 (50.8%)**
Tumor type, n (%)			
Breast, n (%)	68 (23.9%)	1 (1.5%)	67 (98.5%)
Lung, n (%)	51 (18.0%)	41 (80.4%)	10 (19.6%)
Head and Neck, n (%)	11 (3.9%)	10 (90.9%)	1 (9.1%)
Urogynecologic, n (%)	17 (6.0%)	11 (64.7%)	6 (35.3%)
Digestive Tract, n (%)	73 (25.7%)	46 (63.0%)	27 (37.0%)
Other, n (%)	20 (7.0%)	9 (45.0%)	11 (55.0%)
Cancer staging (AJCC 8th Edition)			
I-III, n (%)	76 (31.7%)	27 (35.5%)	49 (64.5%)
IV, n (%)	164 (68.3%)	91 (55.5%)	73 (44.5%)
Cancer Treatment			
ChemoT	160 (51.6%)	76 (47.5%)	84 (52,5%)
ImmunoT	58 (18.7%)	40 (69.0%)	18 (31.0%)
HormonoT	21 (6.8%)	7 (33.3%)	14 (66.7%)
TargetT	57 (18.4%)	22 (38.6%)	35 (61.4%)
Others	14 (4.5%)	1 (7.1 %)	13 (92.9%)
HAEMATOLOGICAL MALIGNANCIES, n (%)	**44 (15.5%)**	**22 (50.0%)**	**22 (50.0%)**
Tumor type, n (%)			
Lymphoid	20 (7.0%)	9 (45.0%)	11 (55.0%)
Leukemia	2 (0.7%)	2 (100%)	0 (0%)
Myeloma	17 (6.0%)	10 (58.8%)	7 (41.2%)
Others	5 (1.8%)	1 (20.0%)	4 (80.0%)
Treatment			
Alkylating antineoplastic agent	16 (20.8%)	6 (37.5%)	10 (62.5%)
Anti-CD20 antibodies	19 (24.7%)	8 (42.1%)	11 (57.9%)
Anthracyclines	10 (13%)	3 (30.0%)	7 (70.0%)
Corticosteroids	22 (28.5%)	11 (50.0%)	11 (50.0%)
Immunomodulatory Drugs (IMiDs)	10 (13%)	7 (70.0%)	3 (30.0%)

2.3 Study Design

To validate this miRNA profile as biomarkers in cancer patients in the context of SARS-CoV-2 vaccination, the selected 7-miRNA profile (hsa-miR-7-5p, hsa-miR-24-3p, hsa-miR-145-5p, hsa-miR-223-3p, hsa-miR-15b-5p, hsa-miR-214 and hsa-miR-98-5p) was quantified in Timepoint −1, Timepoint 1 and Timepoint 2, in cohort 1. Regarding cohort 2 and 3, miRNAs expression was assessed in Timepoint 1 and Timepoint 2. Cellular immune response to COVID-19 vaccination was evaluated in 104 cancer patients following the booster dose of the COVID-19 vaccine through the analysis of IFN-γ production. In Cohort 1, at Timepoint −1, Timepoint 1, and Timepoint 2, complete blood count analysis was performed to calculate systemic inflammatory indices. To study the potential impact of these miRNAs on the development of an effective immune response to COVID-19 vaccination, the expression of all miRNAs was correlated with humoral immunity through the quantification of IgG levels against SARS-CoV-2 Spike (IgGS) and with cellular immunity through IFN-γ detection. Furthermore, to assess their potential role in systemic inflammation, miRNA expression was correlated with systemic inflammatory indices (NLR, PLR, LMR, and SII), providing insight into the relationship between circulating miRNAs and inflammation in cancer patients.

Figure 1- Study Design. Quantification of MiRNAs in cohort 1 in Timepoint −1, Timepoint 1 and Timepoint 2. Regarding cohorts 2 and 3, miRNAs expression was assessed in Timepoint 1 and Timepoint 2. Cellular immune response to COVID-19 vaccination was evaluated in 104 cancer patients following the booster dose of the COVID-19 vaccine through the analysis of IFN-γ production. The expression of all miRNAs was correlated with humoral immunity and with cellular immune response.

2.3. SARS-CoV-2 IgG assay

To study the humoral immune response to SARS-CoV-2 booster vaccine in cancer patients' IgG levels against SARS-CoV-2 Spike (IgGS) and IgG levels against SARS-CoV-2 Nucleocapsid (IgGN) were evaluated in 209 serum of cancer patients and in 138 healthy individuals through the techniques described in a previous study from our research group [14].

2.4 IFN-γ release assay for SARS-CoV-2

Cellular immune response was evaluated using the QuantiFERON SARS-COV-2 kit (Qiagen®). This method is based on the capability of immune cells to produce IFN-γ when stimulated with SARS-COV-2 spike protein antigen. This assay employed four antigen tubes—Ag1 tube, Ag2 tube, Nil tube (negative control), and Mitogen tube (positive control). In the Ag1 tube, the stimulation of CD4+ T cells led to IFN-γ production, while in the Ag2 tube, IFN-γ release was from both stimulated CD4+ and CD8+ T cells. The blood collection tubes were incubated overnight at 37 °C and following the incubation period, the tubes were centrifuged. The plasma sample from the Mitogen tube was defined as an IFN-γ positive control for each specimen tested and the Nil tube was adjusted for background. The QFN SARS-CoV-2 ELISA Immunoassay was conducted, and optical density readings were measured at 450 nm and 630 nm using the Varioskan™ LUX multimode microplate reader. As per the manufacturer's guidelines, an IFN-γ value (Ag1-Nil or Ag2-Nil) of ≥ 0.15 IU/mL is interpreted as a positive response to the antigens. The IFN-γ results were analysed using Qiagen QFN SARS-CoV-2 analysis software version 1.1.0.0. Therefore, these results were achieved using a cutoff point of 0.15 IU/ml to define a Th1 IFN-γ response.

2.5 Haematological parameters and systemic inflammatory indices

Complete blood count analysis, including absolute counts of leukocytes, neutrophils, lymphocytes, monocytes, basophils, and platelets was performed in Timepoint −1, Timepoint 1 and Timepoint 2 in Cohort 1. These haematological parameters were subsequently used to calculate systemic inflammatory

indices, which are commonly employed as surrogate markers of immune and inflammatory status [40, 50]. The neutrophil-to-lymphocyte ratio (NLR) was defined as the ratio of neutrophil to lymphocyte counts, the platelet-to-lymphocyte ratio (PLR) as the ratio of platelet to lymphocyte counts, and the lymphocyte-to-monocyte ratio (LMR) as the ratio of lymphocyte to monocyte counts. The systemic immune-inflammation index (SII) was calculated using the formula: platelet count × neutrophil count / lymphocyte count [50, 51].

2.6 MicroRNA Extraction and cDNA Synthesis

MiRNAs were extracted from the plasma fraction of the patients' samples using the purification system Thermo Scientific™ KingFisher™ Flex extractor (ThermoFisher™ Waltham, MA, USA) through the the MagMAX™ mirVana™ (Thermofisher™ Waltham, MA, USA) Total RNA Isolation Kit. NanoDrop Lite™ spectrophotometer (ThermoFisher™ Waltham, MA, USA) was used to assess the RNA concentration and purity of the samples. cDNA synthesis was proceeded with Taqman® MicroRNA Reverse Transcription kit (Applied Biosystems™ Waltham, MA, USA) along with sequence-specific primers targeting hsa-miR-7-5p, hsa-miR-15b-5p, hsa-miR-24-3p, hsa-miR-145-5p, hsa-miR-98-5p, hsa-miR-223-3p, hsa-miR-214, RNU48, RNU44 and RNU6B. The thermal cycling conditions were as follows: 16 °C for 30 minutes, 42 °C for 60 minutes, and a final step at 85 °C for 10 minutes.

2.7 Quantification of the Selected hsa-miRNAs Profile by Real-Time Quantitative Polymerase Chain Reaction (RT-qPCR)

The quantification of the 7-miRNA was carried out by performing quantitative real-time PCR (qPCR) using the StepOnePlus™ Real-Time PCR System (ThermoFisher™, Waltham, MA, USA). Each reaction was made with a volume of 10 µL including 1X TaqMan™ Fast Advanced Master Mix (Applied Biosystems™), 1X TaqMan® miRNA Expression Assay probes (hsa-miR-7-5p: 005723_mat; hsa-miR-15b-5p: 000390; hsa-miR-24-3p: 000402; hsa-miR-145-5p: 477916_mir; hsa-miR-223-3p: 002295; hsa-miR-214: 002306 and hsa-miR-98-5p: 000577 —Applied Biosystems™), and 2 µL of cDNA. RNU48, RNU44 and RNU6B were used as housekeeping controls for the miRNA expression normalization. The amplification conditions of qPCR started with an initial step of holding stage 95 °C for 20 seconds, followed by 45 cycles of 95 °C for 1 second and 60 °C for 20 seconds. In each run two replicates of each sample were included and a negative control. StepOne™ Software version 2.2 (Applied Biosystems™) was used to Data analysis to apply the same baseline and threshold set for each plate, to generate cycle threshold (Ct) values for all the miRNAs in each sample.

2.7. Data Analysis

MicroRNA expression levels were quantified using TaqMan miRNA expression assays (Applied Biosystems™) by quantitative real-time PCR, which enable the use of the Livak–Schmittgen method to use a comparative quantification. SPSS for Windows (version 29.0) and GraphPad Prism (version 8.0) were used to perform the statistical analysis. Normality of data was assessed using the Shapiro–Wilk and Kolmogorov–Smirnov tests, and group differences were analysed with either an unpaired t-test or the Mann–Whitney U test, as appropriate. Due to their stable expression, RNU48, RNU44, and RNU6B were used as endogenous controls to normalize microRNA levels. The miRNA levels (low versus high) were classified based on the mean value of the ΔCt. Association between microRNAs expression levels and systemic inflammatory indices, including the PLR, SII, NLR and LMR were evaluated using ANOVA tests. Furthermore, Spearman correlation was conducted to study the relationship between miRNA expression patterns and anti-Spike IgG levels.

3. Results

3.1 Cellular immune response to COVID-19 vaccination

Cellular immune response to the vaccination was evaluated through the analysis of IFN-γ levels using the QuantiFERON SARS-COV-2 kit (Qiagen ®). This method is based on the capability of immune cells to produce IFN-γ when stimulated with SARS-COV-2 spike protein antigen. 104 cancer patients' samples were analyzed and we observed that, in the haematological tumor patients' group (N=30), 17 of them exhibited a reactive response and 13 a non-reactive response to the booster dose. In solid tumors (N=74), 47 patients were reactive and 27 non-reactive to the COVID-19 booster vaccination, as it is demonstrated in Figure 2.

As it is represented in Table 2, among the 17 IFN-γ reactive haematological patients, 11 demonstrated an efficient humoral response with a positive production of IgG S and 4 of them didn't have any production of IgG S (data related to the humoral response of these patients are previously published) [14]. Moreover, among the 13 IFN-γ non-reactive haematological patients, 8 were IgG S (+) and 5 IgG S (-) (table 2). Despite the generally impaired humoral immune response in haematological patients, characterized by the incapacity to produce IgG S antibodies, some of them can produce an effective cellular immune response. Regarding the 74 solid tumor patients, the majority were also reactive for IFN-γ (n=47) indicating efficiency in both humoral and cellular immune responses, since they were classified as IFN-reactive and IgG S (+). Between non-reactive patients, 22 were IgG S (+) and 1 IgG S (-).

Table 2-Classification of cancer patients in IFN-γ Reactive or non-reactive and IgG S (+) and IgG S (-).

	IFN-γ	IgG S (+)	IgG S (-)	NA
Hematological Tumors	Reactive (N=17)	11	4	2
	Non Reactive (N=13)	8	5	0
Solid Tumors	Reactive (N=47)	45	0	2
	Non Reactive (N=27)	22	1	4

3.2 MicroRNAs plasmatic Levels in Cancer Patients

The expression of the selected miRNAs was quantified in plasma samples of the three cohorts. Regarding cohort 1 in the 44 solid tumour patients for Timepoint -1 (N=44), Timepoint 1 (N=41) and Timepoint 2 (N=34) it was verified a significant increase of hsa-miR-7-5p, hsa-miR-15b-5p and hsa-miR-98-5p**,** 3 months after boost (Figure 3B, 3C and 3E, respectively). However, all miRNAs levels decrease between the 3- and 6-months post-boost. Hsa-miR-24-3p and hsa-miR-214 also diminish their expression between pre-boost and 6 months after boost (Figure 3A and 3F, respectively).

Figure 3- Quantification of selected miRNAs in the solid tumors of cohort 1 in pre-boost (Preb), 3 months (3M) and 6 months (6M) after booster dose. (A) hsa-miR-24-3p plasmatic expression with mean ± SEM of PreB (N = 44): 10.95 ± 0.35; 3M (N = 41): 11.08 ± 0.30; 6M(N = 34): 9.51 ± 0.26; (B) hsa-miR-7-5p plasmatic expression with mean ± SEM of PreB (N = 44): 0.93 ± 0.33; 3 M (N = 41): 2.17 ± 034; 6 M (N = 34): 0.83 ± 0.30; (C) hsa-miR-15-5p plasmatic expression with mean ± SEM of PreB (N = 44): 8.56 ± 0.33; 3M(N = 41): 9.70 ± 0.32; 6M(N = 34): 8.31 ± 0.26; (D) hsa-miR-145-5p plasmatic expression with mean ± SEM of PreB (N = 44): 5.92 ± 0.37; 3 M (N = 41): 6.84 ± 0.32; 6 M (N = 34): 5.34 ± 0.28; (E) hsa-miR-98-5p plasmatic expression with mean ± SEM of PreB (N = 44): 5.38 ± 0.44; 3 M (N = 41): 7.29 ± 0.42; 6 M (N = 34): 4.37 ± 0.31; (F) hsa-miR-214 plasmatic expression with mean ± SEM of PreB (N = 44): 1.21 ± 0.43; 3 M (N = 41): 1.29 ± 0.33; 6 M (N = 34): -0.21 ± 0.29; (G) hsa-miR-98-5p plasmatic expression with

mean ± SEM of PreB (N = 44): 13.41 ± 0.35; 3 M (N = 41): 14.22 ± 0.31; 6 M (N = 34): 13.12 ± 0.26. *p<0,05; **p<0,01; ***p<0,001

Regarding hematological tumors patients, Timepoint -1 (N=12), Timepoint 1 (N=11) and Timepoint 2 (N=6) (pre-boost group, 3- and 6-months post-boost group respectively) it was only observed significant statistic results for hsa-miR-98-5p which the levels decreased 6 months after boost comparing with the 3 months after boost group (Figure 4E).

Figure 4- Quantification of miRNAs in hematologic tumors of cohort 1 in pre-boost (PreB), 3 months (3M) and 6 months (6M) after booster dose. (A) hsa-miR-24-3p plasmatic expression with mean ± SEM of PreB (N = 12): 10.27 ± 0.58; 3M (N = 11): 11.0 ± 0.63; 6M(N = 6): 9.46 ± 0.16; (B) hsa-miR-7-5p plasmatic expression with mean ± SEM of PreB (N = 12): 0.74 ± 0.43; 3 M (N = 11): 1.54 ± 0.64; 6 M (N = 6): 1.42 ± 1.14; (C) hsa-miR-15-5p plasmatic expression with mean ± SEM of PreB (N = 12): 8.12 ± 0.48; 3M(N = 11): 9.60 ± 0.65; 6M(N = 6): 8.37 ± 0.36; (D) hsa-miR-145-5p plasmatic expression with mean ± SEM of PreB (N = 12): 5.40 ± 0.63; 3 M (N = 11): 6.48 ± 0.58; 6 M (N = 6): 5.03 ± 0.35; (E) hsa-miR-98-5p plasmatic expression with mean ± SEM of PreB (N = 12): 6.13 ± 0.84; 3 M (N = 11): 6.75 ± 0.71; 6 M (N = 6): 3.93 ± 0.48; (F) hsa-miR-214 plasmatic expression with mean ± SEM of PreB (N = 12): 1.95 ± 0.66; 3 M (N = 11): 0.99 ± 0.84; 6 M (N = 6): 1.36 ± 0.61; (G) hsa-miR-223-3p plasmatic expression with mean ± SEM of PreB (N = 12): 13.22 ± 0.67; 3 M (N = 11): 13.78 ± 0.58; 6 M (N = 6): 13.12 ± 0.29. *p<0,05

MiRNAs were also analyzed in all patients of both cohort 1 and cohort 2, -3 and -6 months after booster dose. When the analysis is stratified for solid cancer patients, Timepoint 1 (N=221) and Timepoint 2 (N=161) (3- and 6-months post-boost group respectively), the only observed statistically significant result is the decrease of hsa-miR-7-5p expression 6 months after the booster dose (Figure 5B).

Figure 5- Quantification of miRNAs in solid cancer patients of cohort 1 and cohort 2, 3 months (3M) and 6 months (6M) after booster dose. A) hsa-miR-24-3p plasmatic expression with mean ± SEM of 3M (N = 221): 10.18 ± 0.12; 6M(N = 161): 10.44 ± 0.12; (B) hsa-miR-7-5p plasmatic expression with mean ± SEM of 3 M (N = 221): 0.75 ± 0.14; 6 M (N = 161): 0.26 ± 0.12; (C) hsa-miR-15-5p plasmatic expression with mean ± SEM of 3M(N = 221): 9.05 ± 0.14; 6M(N = 161): 9.13 ± 0.11; (D) hsa-miR-145-5p plasmatic expression with mean ± SEM of 3 M (N = 221): 6.03 ± 0.12; 6 M (N = 161): 5.92 ± 0.12; (E) hsa-miR-98 plasmatic expression with mean ± SEM of 3 M (N = 221): 4.74 ± 0.16; 6 M (N = 161): 4.79 ± 0.12; (F) hsa-miR-214 plasmatic expression with mean ± SEM of 3 M (N = 221): 0.60 ± 1.14; 6M(N = 161): 0.55 ± 0.13; (G) hsa-miR-223-3p plasmatic expression with mean ± SEM of 3 M (N = 221): 13.52 ± 0.12; 6 M (N = 161): 13.82 ± 0.12. **p<0,01.

When focused on hematological cancer patients of both cohort 1 and cohort 2, Timepoint 1 (N=39) and Timepoint 2 (N=29) (3- and 6-months post-boost group respectively) it was observed low expression of hsa-miR-7-5p, hsa-miR-145-5p and hsa-miR-98-5p, 6 months post-boost (Figure 6B, 6D and 6E, respectively).

Figure 6- Quantification of miRNAs in hematological cancer patients of cohort 1 and cohort 2, 3 months (3M) and 6 months (6M) after booster dose. (A) hsa-miR-24-3p plasmatic expression with mean ± SEM of 3M (N = 39): 10.61 ± 0.30; 6M(N = 29): 10.21 ± 0.29; (B) hsa-miR-7-5p plasmatic expression with mean ± SEM of 3 M (N = 39): 1.24 ± 0.34; 6 M (N = 28): 0.12 ± 0.37; (C) hsa-miR-15-5p plasmatic expression with mean ± SEM of 3M(N = 39): 9.46 ± 0.31; 6M(N = 29): 8.87 ± 0.24; (D) hsa-miR-145-5p plasmatic expression with mean ± SEM of 3 M (N = 39): 6.32 ± 0.29; 6 M (N = 29): 5.39 ± 0.29; (E) hsa-miR-98-5p plasmatic expression with mean ± SEM of 3 M (N = 39): 5.16 ± 0.37; 6 M (N = 29): 4.33 ± 0.31; (F) hsa-miR-214 plasmatic expression with mean ± SEM of 3 M (N = 29): 0.92 ± 0.31; 6 M (N = 39): 0.61 ± 0.32;

(G) hsa-miR-223-3p plasmatic expression with mean ± SEM of 3 M (N = 39): 13.87 ± 0.28; 6 M (N = 29): 13.48 ± 0.35. *p<0,05; ** p<0,01.

We also evaluated the expression of all these miRNAs in the cohort 3 in Timepoint 1 (N=86) and Timepoint 2 (N=86), 3- and 6-months post-boost group respectively. In this group of healthy individuals, we observed a decrease of expression 6 months post-boost only in hsa-miR-7-5p and hsa-miR-214 (Figure 7B and 7F). However, hsa-miR-24-3p, hsa-miR-15b-5p, hsa-miR-145-5p and hsa-miR-223-3p demonstrated a significant increase (Figure 7A, 7C, 7D and 7G respectively).

Figure 7- Quantification of miRNAs in healthy individuals, 3 months (3M) and 6 months (6M) after booster dose. A) hsa-miR-24-3p plasmatic expression with mean ± SEM of 3M (N = 82): 8.26 ± 0.24; 6M(N = 84): 9.27 ± 0.21; (B) hsa-miR-7-5p plasmatic expression with mean ± SEM of 3 M (N = 81): 1.06 ± 0.23; 6 M (N = 84): -0.01 ± 0.22; (C) hsa-miR-15-5p plasmatic expression with mean ± SEM of 3M(N = 82): 6.84 ± 0.26; 6M(N = 84): 8.55 ± 0.18; (D) hsa-miR-145-5p plasmatic expression with mean ± SEM of 3 M (N = 82): 4.49 ± 0.22; 6 M (N = 84): 5.40 ± 0.18; (E) hsa-miR-98-5p plasmatic expression with mean ± SEM of 3 M (N = 82): 4.08 ± 0.22; 6 M (N = 84): 4.58 ± 0.20; (F) hsa-miR-214 plasmatic expression with mean ± SEM of 3 M (N = 82): 0.73 ± 0.16; 6M(N = 84): -0.13 ± 0.21; (G) hsa-miR-223-3p plasmatic expression with mean ± SEM of 3 M (N = 82): 11.84 ± 0.25; 6 M (N = 84): 13.12 ± 0.17. **p<0,01; *** p<0,001; **** p<0,0001.

3.3 MiRNAs levels correlation with humoral immune response

In a previous study from our research group, we evaluated the humoral immune response to SARS-CoV-2 booster vaccine in both cancer patients and healthy individuals through the quantification of IgGS and IgGN using the techniques previously described [14]. In that study, the IgGS levels between haematological tumors, solid tumors, and healthy individuals were compared -3 and -6 months after booster dose administration. In timepoint 1 (3 months post-boost), it was observed that the antibody levels are higher in healthy individuals (332.9 ± 274.5 U/mL) comparing with solid tumors patients (190.6 ± 257.9 U/mL) and with haematologic tumors (114.2 ± 233.3 U/mL). Also, patients with solid tumors had higher IgGS levels comparing with haematological tumors. Relating to timepoint 2 (6 months post-boost), the antibody levels were higher in healthy individuals (361.76 ± 291.6 U/mL) comparing with solid tumors (153.8 ± 242.8 U/mL) and with haematological tumors patients (143.2 ± 246.2 U/mL) [14]. To understand if there is some association between the humoral immune response and the panel of miRNAs it was performed a Spearman correlation between miRNAs expression and IgG S levels in solid and hematological cancer patients -3- and -6-months post-boost (table 3 and table 4, respectively). Regarding to hsa-miR-7-5p, it was verified a significant positive Spearman correlation between its expression and IgG S levels (p=0.011) 6 months post boost in solid cancer patients. This result indicates that higher levels of miR-7-5p expression are associated with higher IgG S levels.

Table 3- Spearman correlation between IgG S and miRNAs expression 3 months and 6 months post boost in solid cancer patients.

		miR-24-3p	miR-7-5p	miR-15b-5p	miR-145-5p	miR-98-5p	miR-214	miR-223-3p
3 Months	Spearman rho	-0.017	-0.100	0.012	-0.006	0.060	-0.115	-0.021
	p-value	0.797	0.138	0.858	0.926	0.373	0.088	0.753
	N	221	221	221	221	221	221	221
6 Months	Spearman rho	-0.108	**0.201**	-0.110	-0.123	-0.055	-0.065	-0.115
	p-value	0.173	**0.011**	0.166	0.119	0.490	0.413	0.147
	N	161	**161**	161	161	161	161	161

Table 4- Spearman correlation between IgG S and miRNAs expression 3 months and 6 months post boost in hematological cancer patients.

		miR-24-3p	miR-7-5p	miR-15b-5p	miR-145-5p	miR-98-5p	miR-214	miR-223-3p
3 Months	Spearman rho	-0.046	0.003	0.052	0.048	-0.230	-0.006	0.048
	p-value	0.783	0.986	0.752	0.771	0.159	0.972	0.771
	N	39	39	39	39	39	39	39
6 Months	Spearman rho	0.199	-0.292	0.165	0.109	0.165	-0.341	0.210
	p-value	0.300	0.131	0.392	0.573	0.394	0.070	0.274
	N	29	28	29	29	29	29	29

3.4 MiRNAs levels correlation with cellular immune response

To further analyze the possible impact of miRNAs expression and the cellular immune response, it was investigated the association between the expression of the selected miRNAs and the IFN-γ response. As it is represented in Figure 8, patients IFN-γ non-reactive demonstrated higher levels of hsa-miR-7-5p than patients IFN-γ positive. The other miRNAs didn't demonstrate a significant association.

Figure 8- Quantification of miRNA expression in IFN (-) and IFN (+) patients. *p<0,05.

4. MiRNAs levels correlation with systemic inflammatory indices

Table 5- MiRNAs expression levels (low versus high) association with systemic inflammatory indices (PLR, SII, NLR and LMR).

		PLR (Mean ± SEM)	*P*	SII (Mean ± SEM)	*P*	NLR (Mean ± SEM)	*P*	LMR (Mean ± SEM)	*P*
hsa-miR-24-3p	Low	158,6±8,68	**0,002**	666,1±79,4	0,140	3,22±0,32	0,431	2,830±0,18	0,808
	High	213,9±14,5		919,1±145,7		3,70±0,50		2,91±0,28	
hsa-miR-7-5p	Low	161,9±10,2	**0,029**	570,1±46,6	**0,039**	2,86±0,19	0,122	2,75±0,22	0,592
	High	201,7±12,4		931,0±129,7		3,83±0,46		2,94±0,23	
hsa-miR-98-5p	Low	178,7±13,4	0,427	671,8±92,2	0,209	3,54±0,54	0,846	2,78±0,22	0,681
	High	192,9±11,7		887,8±128,9		3,42±0,35		2,93±0,24	
hsa-miR-15b-5p	Low	177,7±12,1	0,325	720,3±95,1	0,396	3,64±0,53	0,606	2,77±0,20	0,579
	High	195,1±12,7		864,9±135,0		3,32±0,33		2,95±0,26	
hsa-miR-223-3p	Low	178,2±10,8	0,267	825,2±132,7	0,716	3,78±0,48	0,252	2,79±0,18	0,625
	High	197,9±14,6		763,0±94,6		3,08±0,31		2,96±0,30	
hsa-miR-145-5p	Low	169,0±9,9	0,053	666,0±66,1	0,141	3,23±0,31	0,449	2,74±0,17	0,480
	High	203,1±14,0		915,4±148,3		3,68±0,50		2,98±0,28	
hsa-miR-214	Low	186,6±11,3	0,963	913,4±139,1	0,154	3,67±0,34	0,484	2,56±0,14	0,051
	High	187,4±13,8		671,8±84,6		3,25±0,51		3,21±0,31	

To investigate the potential impact of miRNAs expression on systemic inflammation in cancer patients, circulating miRNAs levels were stratified into low and high expression groups and associated with systemic inflammatory indices as biomarkers of inflammation, including PLR, SII, NLR, and LMR. As shown in Table 5, patients with high expression of hsa-miR-24-3p exhibited significantly higher PLR compared with those with low expression, while high expression of hsa-miR-7-5p was significantly associated with increased PLR and SII, suggesting a potential correlation between these miRNAs and systemic inflammatory status. Patients with higher expression of hsa-miR-145-5p and hsa-miR-214 tended to show increased PLR (hsa-miR-145-5p) and borderline higher LMR (hsa-miR-214), indicating a tendency toward a potential association between these miRNAs and systemic inflammatory markers. In contrast, no significant associations were observed between the expression levels of hsa-miR-98-5p, hsa-miR-15b-5p, or hsa-miR-223-3p and any of the evaluated inflammatory indices (PLR, SII, NLR, or LMR).

5. Discussion

Cancer patients are more vulnerable to severe outcomes from COVID-19. Identifying biomarkers that predict which cancer patients are at high risk of severe COVID-19 and the real effectiveness of the immune responses after booster vaccines could aid in prioritizing early clinical interventions in the future [52]. MiRNAs located in genomic regions linked to SARS-CoV-2 infection may serve as potential biomarkers for predicting clinical outcomes [23]. Several studies, including one previously published by our group, evaluate the humoral immunity indicating that healthy individuals exhibited a more robust immune response to the SARS-CoV-2 booster vaccine compared to cancer patients [14, 53, 54]. Within the cancer patient group, those with solid tumors responded better to the vaccine than those with haematological

malignancies, and among the haematological patients, higher titers of antibodies were detected in patients with multiple myeloma [14]. Moreover, chemotherapy and febrile neutropenia risk are clinical factors that further diminish the effectiveness of booster doses. In our previous study, it has been observed that patients with solid tumors undergoing chemotherapy, particularly those on high-risk febrile neutropenia regimens, exhibit significantly reduced IgG S levels [14]. Alongside the humoral immune response, it is crucial to thoroughly understand the cellular immune response to COVID-19 vaccination in these patients [14-16]. Indeed, despite the inefficient humoral response in haematological cancer patients of our study, some of them were protected by cellular immunity, as 17 of these patients exhibited a reactive response. Our results align with Body et al. study, which demonstrated that a T-cell response is detected in most patients regardless of their antibody response [55]. In this study, 65 % of patients receiving B-cell depleting therapies did not develop an efficient antibody response after a three-dose primary course. Nevertheless, most of these patients had T cell response as measured by IFN-γ [55]. Additionally, Nguyen et al. showed that haematology patients who fail to generate memory B cell responses post-vaccination can produce robust SARS-CoV-2-specific T cell immunity [56]. Relating to solid cancer patients included in our study, most appear to have dual protection, exhibiting both efficient humoral and cellular immune responses. Based on these findings, it is important to prioritize booster vaccination for individuals at high risk of immunological failure, such as cancer patients, as most can benefit from the protection conferred by the activation of humoral and/or cellular immune responses [57]. However, there are specific cases where vaccination is not sufficiently effective, and these patients may eventually benefit from emerging therapies. We evaluated the expression of miRNAs described as able to target Spike and TMPRSS2 mRNA, to determine whether their expression could impact the efficiency of the immune response to vaccination. Regarding solid cancer patients of cohort 1, the expression of hsa-miR-7-5p, hsa-miR-15b-5p and hsa-miR-98-5p increased 3 months after boost administration and, 6 months post-boost, the expression returned to approximately the same levels before the booster dose. Since these miRNAs can inhibit viral translation by targeting the mRNA of the Spike protein, the increase of these molecules may be the response to attenuate the inflammation caused by COVID-19 vaccination. This could suppress the transcription and translation involved in viral replication and protein synthesis. In haematological patients, the same biological tendency was observed for hsa-miR-98-5p whose levels decreased at 6 months compared with the 3 months post-boost. When analysing all patients in cohort 1 and cohort 2, at both -3 and -6 months post-boost, it was observed a decrease in hsa-miR-7 in solid cancer patients, while both hsa-miR-98 and hsa-miR-7 levels decreased in haematological cancer patients 6 months post-boost. After this period the inflammation triggered by the COVID-19 vaccination diminishes so the expression of these miRNAs may also decline. Regarding hsa-miR-7-5p, we observed a significant correlation between its expression and antibody levels in solid cancer patients. Therefore, hsa-miR-7-5p appears to have a direct role in regulating humoral immunity. This conclusion is supported by a significant positive Spearman correlation between hsa-miR-7-5p expression and IgG S levels, indicating that higher levels of hsa-miR-7-5p expression are associated with higher IgG S levels. This result supports the hypothesis that these variations reflect a potential adaptive response of the organism to an exacerbation of viral mRNA elements, suggesting that the immune system is reacting to the elevated presence of viral proteins. Additionally, IFN-γ non-reactive patients demonstrated higher levels of hsa-miR-7-5p compared to IFN-γ positive patients, which may be a clue about the potential impact of this miRNA on the cellular immune response. Therefore, hsa-miR-7-5p stands out for its potential influence on both humoral and cellular immune responses, warranting further investigation into its mechanisms and therapeutic applications. On the other hand, healthy individuals exhibited a distinct biological trend, with certain miRNAs showing significant increases. In these individuals, IgG spike levels were higher compared to cancer patients at all timepoints. Consequently, 6 months post-booster dose, the mRNA levels of the spike protein remained elevated. Based on this, it can be hypothesized that the increased expression of these miRNAs may reflect a response mechanism to the

elevated presence of viral mRNA, acting as part of the immune reaction to the higher levels of viral protein to normalize immunopathogenesis. We also evaluated the potential impact of miRNAs expression on systemic inflammation in cancer patients', post-vaccination, given their critical role in disease progression and clinical outcomes. To this end, we stratified patients into low and high miRNA expression groups and examined their associations with systemic inflammatory indices, including PLR, SII, NLR, and LMR. While previous studies have reported links between certain miRNAs and inflammatory markers, to our knowledge, this is the first study that investigate this specific panel of miRNAs in relation to these systemic inflammatory indices in a cancer context after the administration of a vaccine during active treatment [37, 39]. Notably, patients with high expression of hsa-miR-24-3p exhibited significantly higher PLR, whereas elevated hsa-miR-7-5p expression was associated with both increased PLR and SII. These findings suggest that these miRNAs may contribute to systemic inflammatory status in cancer patients, highlighting their potential as biomarkers of inflammation and underscoring a possible role in inflammation driven tumour progression. Indeed, previous studies have demonstrated that overexpression of hsa-miR-7-5p and hsa-miR-24-3p are correlated with human inflammatory diseases. For instance, elevated hsa-miR-7-5p levels have been reported in patients with type 1 diabetes, where they positively correlated with systemic inflammatory biomarkers such as C-reactive protein and CXCL-10, reflecting heightened inflammatory status [58]. Similarly, altered hsa-miR-7-5p expression has been observed in idiopathic inflammatory myopathies, suggesting a role in systemic immune activation [59]. Regarding hsa-miR-24-3p, its expression is increased in inflamed colonic epithelial cells from patients with ulcerative colitis and is detectable in circulation, where it associates with inflammatory activity [60]. Importantly, our findings demonstrate that these miRNAs are associated with systemic inflammation in the cancer context, providing novel evidence of their potential role in modulating cancer-related inflammatory responses. In particular, the association of hsa-miR-7-5p with both PLR and SII, together with its correlation with humoral and cellular immune responses observed in our analyses, suggests that miR-7-5p may represent a central biomarker integrating systemic inflammation and immune dysregulation in cancer, serving as a key biomarker in cancer patients. Taken together, our findings suggest that hsa-miR-7-5p may have potential as a biomarker to stratify cancer patients according to their immune response to COVID-19 vaccination. In solid tumour patients, higher hsa-miR-7-5p levels were associated with increased IgG S titres, reflecting effective humoral immunity. This upregulation likely represents a regulatory response to viral mRNA, helping to control spike protein levels. While it supports effective short term immunity, it may limit long-term immune persistence, highlighting the importance of continued booster vaccination. Haematological patients with low antibody production and high hsa-miR-7-5p levels could be considered poor responders, as this elevated expression is associated with IFN-γ non-reactivity, potentially requiring intensified monitoring and alternative preventive strategies. Furthermore, the association of elevated hsa-miR-7-5p with systemic inflammatory biomarkers suggests that persistently high levels may indicate a pro-inflammatory state and worse long-term outcomes. However, further investigation into the regulatory mechanisms of these miRNAs is essential to fully understand their impact on immune response. Validating these miRNAs as biomarkers could enhance personalized medicine strategies, enabling more targeted management of high-risk cancer patients in the context of COVID-19 vaccination.

6. Conclusion

This study highlights the critical need to prioritize COVID-19 booster vaccinations for cancer patients, particularly those at high risk of immunological failure. Our findings underscore the complexity of immune responses in cancer patients, revealing that even with an impaired humoral response, specially observed in haematological malignancies, cellular immunity can provide significant protection against severe COVID-19 outcomes. This highlights the importance of assessing both humoral and cellular responses when evaluating vaccine efficacy in immunocompromised populations. Moreover, the identification of specific

microRNAs, as possible modulators of immune signalling pathways following vaccination, provides new insights into the mechanisms of vaccine-induced immunity. Notably, hsa-miR-7-5p demonstrate dual influence on both humoral and cellular immune responses warrants further investigation into it’s potential in therapeutic applications. Moreover, both hsa-miR-7-5p and hsa-miR-24-3p demonstrated an impact on systemic inflammation in cancer patients, further supporting their role as inflammation associated biomarkers. Notably, hsa-miR-7-5p appears to play a particularly important role, as it was consistently associated with humoral and cellular immune responses as well as with systemic inflammatory status, emphasizing its potential as a key biomarker of immune and inflammatory regulation in cancer patients. Incorporating these miRNAs into personalized medicine strategies could enable more targeted management of high-risk cancer patients, optimizing vaccination schedules and enhancing protective immunity.

Declarations

Ethics Approval and Consent to Participate

This project received approval from the Ethics Committee of the Portuguese Oncology Institute of Porto (IPO-Porto) (CES IPO: 286/021). All the patients enrolled in the study signed a written informed consent, under the principles of the Helsinki Declaration. The informed consent form is provided in the supplementary materials. Clinical trial number: not applicable.

Declaration of Competing Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Author Contribution

Conceptualization, F.D. and T.R.D.; Data curation, T.R.D., F.D., P.C., M.S.-P., M.M., L.R. and J.M.M.; Formal analysis, T.R.D., A.L.T., and F.D.; Funding acquisition, R.M. and J.O.; Investigation, F.D., T.R.D., and B.A.; Methodology, B.A. and T.R.D.; Project administration, R.M. and J.O.; Supervision, F.D., A.L.T. and R.M.; Validation, F.D. and R.M.; Visualization, F.D., R.M. and J.O.; Writing—original draft, T.R.D.; Writing—review and editing, F.D. and R.M. All authors have read and agreed to the published version of the manuscript.

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Data Availability

The data presented in this study are available on request from the corresponding author since these data are included in a larger ongoing project.

References

1. Cucinotta, D. & Vanelli, M. WHO Declares COVID-19 a Pandemic. *Acta Biomed.* **91** (1), 157–160 (2020).
2. Acuti Martellucci, C. et al. SARS-CoV-2 pandemic: An overview. *Adv. Biol. Regul.* **77**, 100736 (2020).
3. Rébé, C. & Ghiringhelli, F. Cytotoxic effects of chemotherapy on cancer and immune cells: how can it be modulated to generate novel therapeutic strategies? *Future Oncol.* **11** (19), 2645–2654 (2015).
4. Pfortmueller, C. A. et al. COVID-19-associated acute respiratory distress syndrome (CARDS): Current knowledge on pathophysiology and ICU treatment - A narrative review. *Best Pract. Res. Clin. Anaesthesiol.* **35** (3), 351–368 (2021).
5. Jawad, B. et al. *Computational Design of Miniproteins as SARS-CoV-2 Therapeutic Inhibitors*. *Int. J. Mol. Sci.*, **23**(2). (2022).
6. Yang, J. et al. A vaccine targeting the RBD of the S protein of SARS-CoV-2 induces protective immunity. *Nature* **586** (7830), 572–577 (2020).
7. Chavda, V. P. et al. *mRNA-Based Vaccines and Therapeutics for COVID-19 and Future Pandemics*. *Vaccines (Basel)*, **10**(12). (2022).
8. Fendler, A. et al. COVID-19 vaccines in patients with cancer: immunogenicity, efficacy and safety. *Nat. Rev. Clin. Oncol.* **19** (6), 385–401 (2022).
9. Packer, M. et al. A novel mechanism for the loss of mRNA activity in lipid nanoparticle delivery systems. *Nat. Commun.* **12** (1), 6777 (2021).
10. Malard, F. et al. Weak immunogenicity of SARS-CoV-2 vaccine in patients with hematologic malignancies. *Blood Cancer J.* **11** (8), 142 (2021).
11. Uslu, G. H., Rakici Aysegul, S. Y. & Yazici, Z. A. COVID-19 vaccine immunity in oncology patients. *Bratisl Lek Listy*. **124** (3), 187–192 (2023).
12. Tran, S., Truong, T. H. & Narendran, A. Evaluation of COVID-19 vaccine response in patients with cancer: An interim analysis. *Eur. J. Cancer*. **159**, 259–274 (2021).
13. Cohen, I. et al. The Humoral Response of Patients With Cancer to Breakthrough COVID-19 Infection or the Fourth BNT162b2 Vaccine Dose. *Oncologist* **28** (4), e225–e227 (2023).
14. Oliveira, J. et al. *Humoral Response to SARS-CoV-2 Vaccine-Boost in Cancer Patients: A Case Series from a Southern European Cancer Center*. *Vaccines (Basel)*, **12**(11). (2024).
15. Mansi, L. et al. Study of the SARS-CoV-2-specific immune T-cell responses in COVID-19-positive cancer patients. *Eur. J. Cancer*. **150**, 1–9 (2021).
16. Scurr, M. J. et al. Whole blood-based measurement of SARS-CoV-2-specific T cells reveals asymptomatic infection and vaccine immunogenicity in healthy subjects and patients with solid-organ cancers. *Immunology* **165** (2), 250–259 (2022).

17. Krüttgen, A. et al. Evaluation of the QuantiFERON SARS-CoV-2 interferon- release assay in mRNA-1273 vaccinated health care workers. *J. Virol. Methods*. **298**, 114295 (2021).
18. Safont, G. et al. Measurement of IFN-γ and IL-2 for the assessment of the cellular immunity against SARS-CoV-2. *Sci. Rep.* **14** (1), 1137 (2024).
19. Rydyznski Moderbacher, C. et al. Antigen-Specific Adaptive Immunity to SARS-CoV-2 in Acute COVID-19 and Associations with Age and Disease Severity. *Cell* **183** (4), 996–1012e19 (2020).
20. Wang, X. et al. Vaccine-induced protection against SARS-CoV-2 requires IFN-γ-driven cellular immune response. *Nat. Commun.* **14** (1), 3440 (2023).
21. Primorac, D. et al. Adaptive Immune Responses and Immunity to SARS-CoV-2. *Front. Immunol.* **13**, 848582 (2022).
22. Abedi, F. et al. MicroRNAs and SARS-CoV-2 life cycle, pathogenesis, and mutations: biomarkers or therapeutic agents? *Cell. Cycle*. **20** (2), 143–153 (2021).
23. Almeida, B. et al. *Plasma EV-miRNAs as Potential Biomarkers of COVID-19 Vaccine Immune Response in Cancer Patients*. *Vaccines (Basel)*, (2024). 12(8).
24. Visacri, M. B. et al. Role of miRNAs as biomarkers of COVID-19: a scoping review of the status and future directions for research in this field. *Biomark. Med.* **15** (18), 1785–1795 (2021).
25. Tang, H. et al. The noncoding and coding transcriptional landscape of the peripheral immune response in patients with COVID-19. *Clin. Transl Med.* **10** (6), e200 (2020).
26. Wang, Y. et al. Decreased inhibition of exosomal miRNAs on SARS-CoV-2 replication underlies poor outcomes in elderly people and diabetic patients. *Signal. Transduct. Target. Ther.* **6** (1), 300 (2021).
27. Almeida, B. et al. *MicroRNAs Derived from Extracellular Vesicles: Keys to Understanding SARS-CoV-2 Vaccination Response in Cancer Patients?*. *Cancers (Basel)*, **15**(16). (2023).
28. Saha, I., Ghosh, N. & Plewczynski, D. Identification of Human miRNA Biomarkers Targeting the SARS-CoV-2 Genome. *ACS Omega*. **7** (50), 46411–46420 (2022).
29. Vaddadi, K. et al. Cellular microRNAs target SARS-CoV-2 spike protein and restrict viral replication. *Am. J. Physiol. Cell. Physiol.* **325** (2), C420–c428 (2023).
30. Lee, Y. S. & Dutta, A. *MicroRNAs cancer Annu. Rev. Pathol.*, **4**: 199–227. (2009).
31. Deng, S. et al. Expression of miR-214 in pancreatic cancer and its effect on the biological function of pancreatic cancer cells. *J. buon*. **26** (3), 1111–1120 (2021).
32. Chen, Y. N. et al. MicroRNA let–7d–5p rescues ovarian cancer cell apoptosis and restores chemosensitivity by regulating the p53 signaling pathway via HMGA1. *Int. J. Oncol.* **54** (5), 1771–1784 (2019).
33. Świtlik, W. Z. & Szemraj, J. Circulating miRNAs as non-invasive biomarkers for non-small cell lung cancer diagnosis, prognosis and prediction of treatment response. *Postepy Hig Med. Dosw (Online)*. **71** (0), 649–662 (2017).
34. Shahverdi, M. & Darvish, M. Exosomal microRNAs: A Diagnostic and Therapeutic Small Bio-molecule in Esophageal Cancer. *Curr. Mol. Med.* **23** (4), 312–323 (2023).
35. Dias, T. R. et al. *MicroRNAs as Potential Tools for Predicting Cancer Patients' Susceptibility to SARS-CoV-2 Infection and Vaccination Response*. *Cells*, **11**(15). (2022).
36. Kaur, T. et al. Tmprss2 specific miRNAs as promising regulators for SARS-CoV-2 entry checkpoint. *Virus Res.* **294**, 198275 (2021).
37. Chakraborty, C. et al. The Interplay among miRNAs, Major Cytokines, and Cancer-Related Inflammation. *Mol. Ther. Nucleic Acids*. **20**, 606–620 (2020).
38. Das, K. & Rao, L. V. M. *The Role of microRNAs in Inflammation*. *Int. J. Mol. Sci.*, **23**(24). (2022).

39. Tili, E., Michaille, J. J. & Croce, C. M. MicroRNAs play a central role in molecular dysfunctions linking inflammation with cancer. *Immunol. Rev.* **253** (1), 167–184 (2013).
40. Martinez, J. M. et al. Diagnostic accuracy of neutrophil-to-lymphocyte ratio, platelet-to-lymphocyte ratio, and neutrophil-lymphocyte-to-platelet ratio biomarkers in predicting bacteremia and sepsis in immunosuppressive patients with cancer: literature review. *Porto Biomed. J.* **9** (3), 254 (2024).
41. Wang, R. H. et al. The clinical value of neutrophil-to-lymphocyte ratio (NLR), systemic immune-inflammation index (SII), platelet-to-lymphocyte ratio (PLR) and systemic inflammation response index (SIRI) for predicting the occurrence and severity of pneumonia in patients with intracerebral hemorrhage. *Front. Immunol.* **14**, 1115031 (2023).
42. Chen, J. H. et al. Systemic immune-inflammation index for predicting prognosis of colorectal cancer. *World J. Gastroenterol.* **23** (34), 6261–6272 (2017).
43. Liu, K. et al. *Systemic immune-inflammatory biomarkers (SII, NLR, PLR and LMR) linked to non-alcoholic fatty liver disease risk*. *Front. Immunol.*, Volume 15–2024. (2024).
44. Jiang, Y., Zang, M. & Li, S. Serum PLR and LMR in Behçet's disease: Can they show the disease activity? *Med. (Baltim).* **96** (21), e6981 (2017).
45. Li, J. et al. Association of the systemic immuno-inflammation index, neutrophil-to-lymphocyte ratio, and platelet-to-lymphocyte ratio with diabetic microvascular complications. *Front. Endocrinol. (Lausanne).* **15**, 1367376 (2024).
46. Zhang, B. et al. The serum platelet-to-lymphocyte ratio as a predictor of outcomes in bladder cancer patients: an updated meta-analysis. *Sci. Rep.* **15** (1), 39502 (2025).
47. Carollo, C. et al. *Silent Inflammation, Loud Consequences: Decoding NLR Across Renal, Cardiovascular and Metabolic Disorders*. *Int. J. Mol. Sci.*, **26**(17). (2025).
48. Xia, Y. et al. *Systemic Immune Inflammation Index (SII), System Inflammation Response Index (SIRI) and Risk of All-Cause Mortality and Cardiovascular Mortality: A 20-Year Follow-Up Cohort Study of 42,875 US Adults*. *J. Clin. Med.*, **12**(3). (2023).
49. Song, Y. et al. Systemic immune-inflammation index is associated with hepatic steatosis: Evidence from NHANES 2015–2018. *Front. Immunol.* **13**, 1058779 (2022).
50. Marques, P. et al. Serum Inflammation-based Scores in Endocrine Tumors. *J. Clin. Endocrinol. Metabolism*. **106** (10), e3796–e3819 (2021).
51. Zhang, J. et al. Single and combined use of the platelet-lymphocyte ratio, neutrophil-lymphocyte ratio, and systemic immune-inflammation index in gastric cancer diagnosis. *Front. Oncol.* **13**, 1143154 (2023).
52. Anfossi, S. et al. MicroRNAs are enriched at COVID-19 genomic risk regions, and their blood levels correlate with the COVID-19 prognosis of cancer patients infected by SARS-CoV-2. *Mol. Cancer*. **23** (1), 235 (2024).
53. Chumsri, S. et al. Humoral Responses After SARS-CoV-2 mRNA Vaccination and Breakthrough Infection in Cancer Patients. *Mayo Clin. Proc. Innov. Qual. Outcomes*. **6** (2), 120–125 (2022).
54. Campagna, R. et al. COVID-19 vaccination in cancer patients: Immune responses one year after the third dose. *Vaccine* **42** (10), 2687–2694 (2024).
55. Body, A. et al. *Comprehensive humoral and cellular immune responses to COVID-19 vaccination in adults with cancer*. *Vaccine*, : p. 126547. (2024).
56. Nguyen, T. H. O. et al. Robust SARS-CoV-2 T cell responses with common TCRαβ motifs toward COVID-19 vaccines in patients with hematological malignancy impacting B cells. *Cell. Rep. Med.* **4** (4), 101017 (2023).

57. Uaprasert, N. et al. Immunogenicity and risks associated with impaired immune responses following SARS-CoV-2 vaccination and booster in hematologic malignancy patients: an updated meta-analysis. *Blood Cancer J.* **12** (12), 173 (2022).
58. Bakhashab, S. et al. *Decoding of miR-7-5p in Colony Forming Unit-Hill Colonies as a Biomarker of Subclinical Cardiovascular Disease-A MERIT Study. Int. J. Mol. Sci.*, **24**(15). (2023).
59. Yu, L. et al. hsa-miR-7 Is a Potential Biomarker for Idiopathic Inflammatory Myopathies with Interstitial Lung Disease in Humans. *Ann. Clin. Lab. Sci.* **48** (6), 764–769 (2018).
60. Soroosh, A. et al. miR-24 Is Elevated in Ulcerative Colitis Patients and Regulates Intestinal Epithelial Barrier Function. *Am. J. Pathol.* **189** (9), 1763–1774 (2019).

Figures

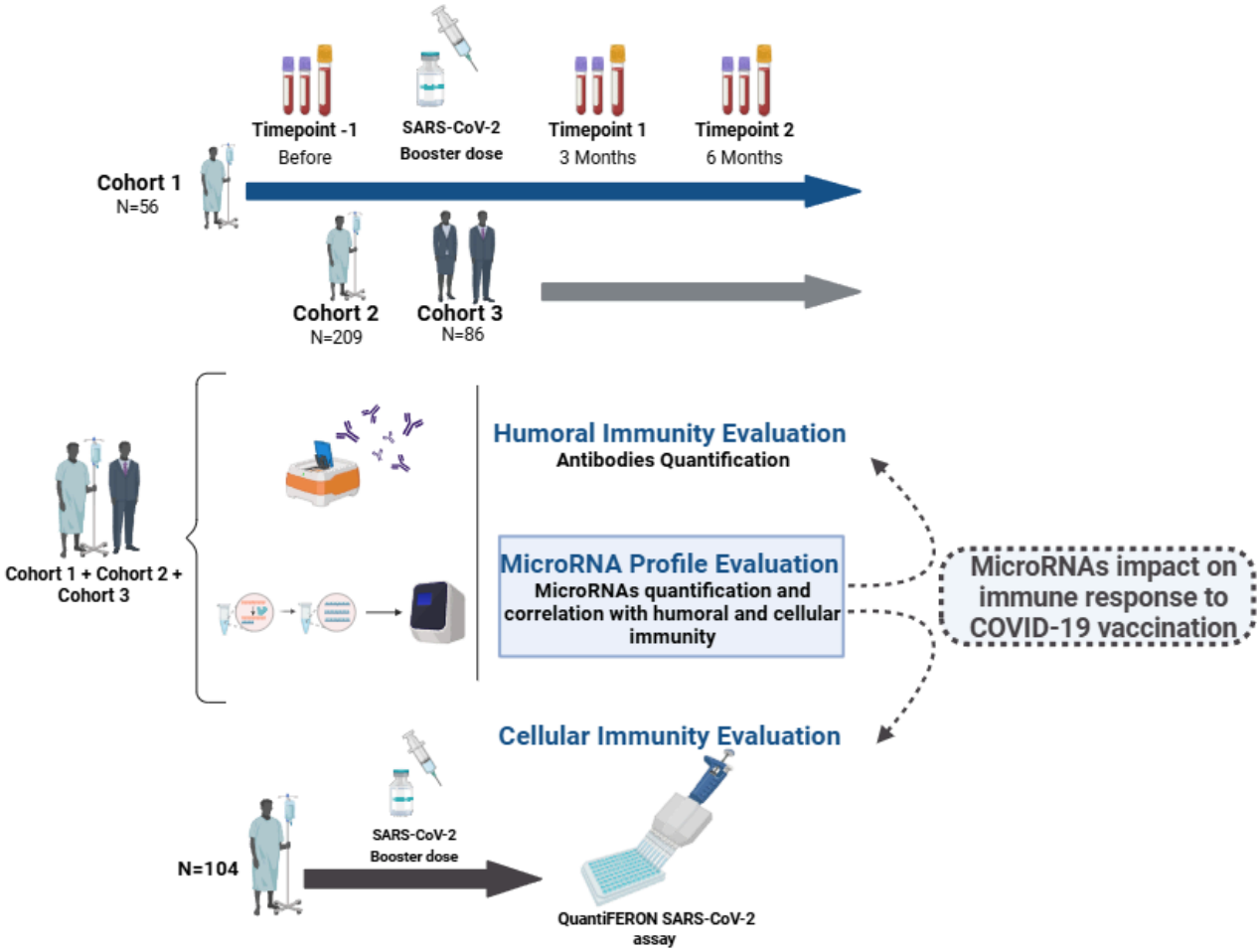


Figure 1

Study Design. Quantification of MiRNAs in cohort 1 in Timepoint −1, Timepoint 1 and Timepoint 2. Regarding cohorts 2 and 3, miRNAs expression was assessed in Timepoint 1 and Timepoint 2. Cellular immune response to COVID-19 vaccination was evaluated in 104 cancer patients following the booster dose of the COVID-19 vaccine through the analysis of IFN-γ production. The expression of all miRNAs was correlated with humoral immunity andwith cellular immune response.

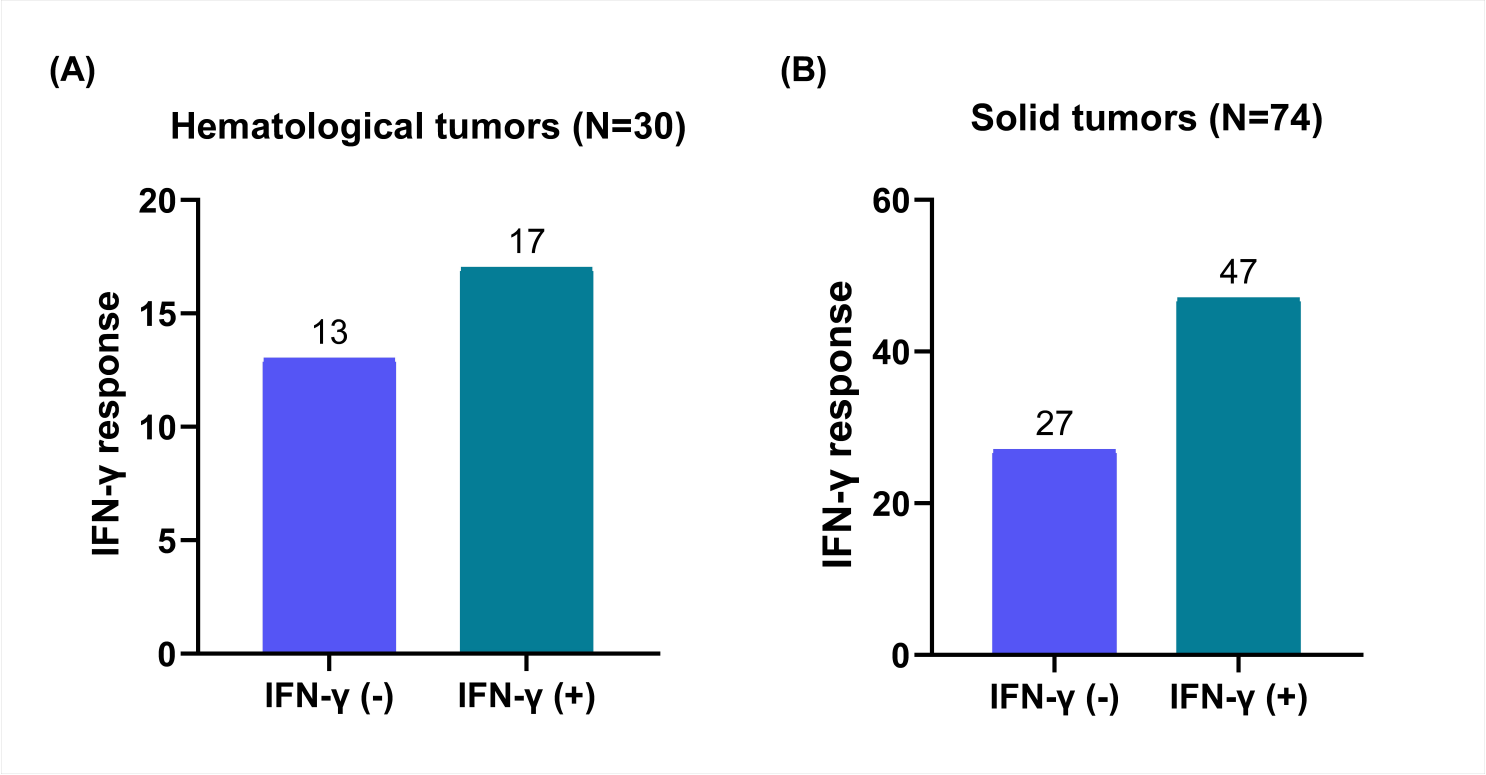


Figure 2

Evaluation of IFN-γ levels in solid and hematological tumors. Quantification of IFN-γ levels in 30 hematological tumors (17 IFN-γ (+) and 13 IFN-γ (-)) and 74 solid tumors (47 IFN-γ (+) and 27 IFN-γ (-)).

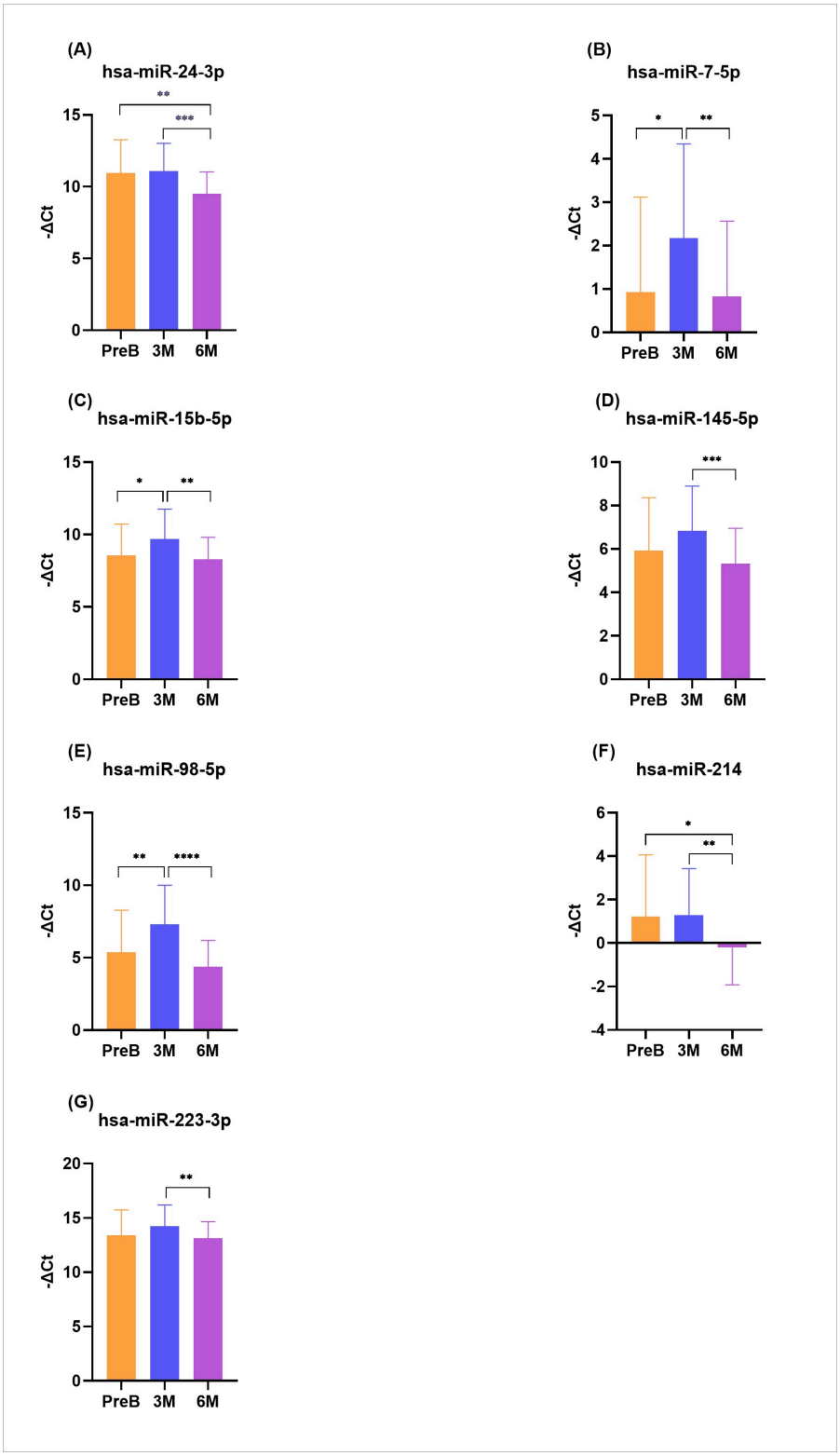


Figure 3

Quantification of selected miRNAs in the solid tumors of cohort 1 in pre-boost (Preb), 3 months (3M) and 6 months (6M) after booster dose. (A) hsa-miR-24-3p plasmatic expression with mean ± SEM of PreB (N = 44): 10.95 ± 0.35; 3M (N = 41): 11.08 ± 0.30; 6M(N = 34): 9.51 ± 0.26; (B) hsa-miR-7-5p plasmatic expression with mean ± SEM of PreB (N = 44): 0.93 ± 0.33; 3 M (N = 41): 2.17 ± 034; 6 M (N = 34): 0.83 ± 0.30; (C) hsa-miR-15-5p plasmatic expression with mean ± SEM of PreB (N = 44): 8.56 ± 0.33; 3M(N = 41): 9.70 ± 0.32; 6M(N = 34): 8.31 ± 0.26; (D) hsa-miR-145-5p plasmatic expression with mean ± SEM of

PreB (N = 44): 5.92 ± 0.37; 3 M (N = 41): 6.84 ± 0.32; 6 M (N = 34): 5.34 ± 0.28; (E) hsa-miR-98-5p plasmatic expression with mean ± SEM of PreB (N = 44): 5.38 ± 0.44; 3 M (N = 41): 7.29 ± 0.42; 6 M (N = 34): 4.37 ± 0.31; (F) hsa-miR-214 plasmatic expression with mean ± SEM of PreB (N = 44): 1.21 ± 0.43; 3 M (N = 41): 1.29 ± 0.33; 6 M (N = 34): -0.21 ± 0.29; (G) hsa-miR-98-5p plasmatic expression with mean ± SEM of PreB (N = 44): 13.41 ± 0.35; 3 M (N = 41): 14.22 ± 0.31; 6 M (N = 34): 13.12 ± 0.26. *p<0,05; **p<0,01; ***p<0,001

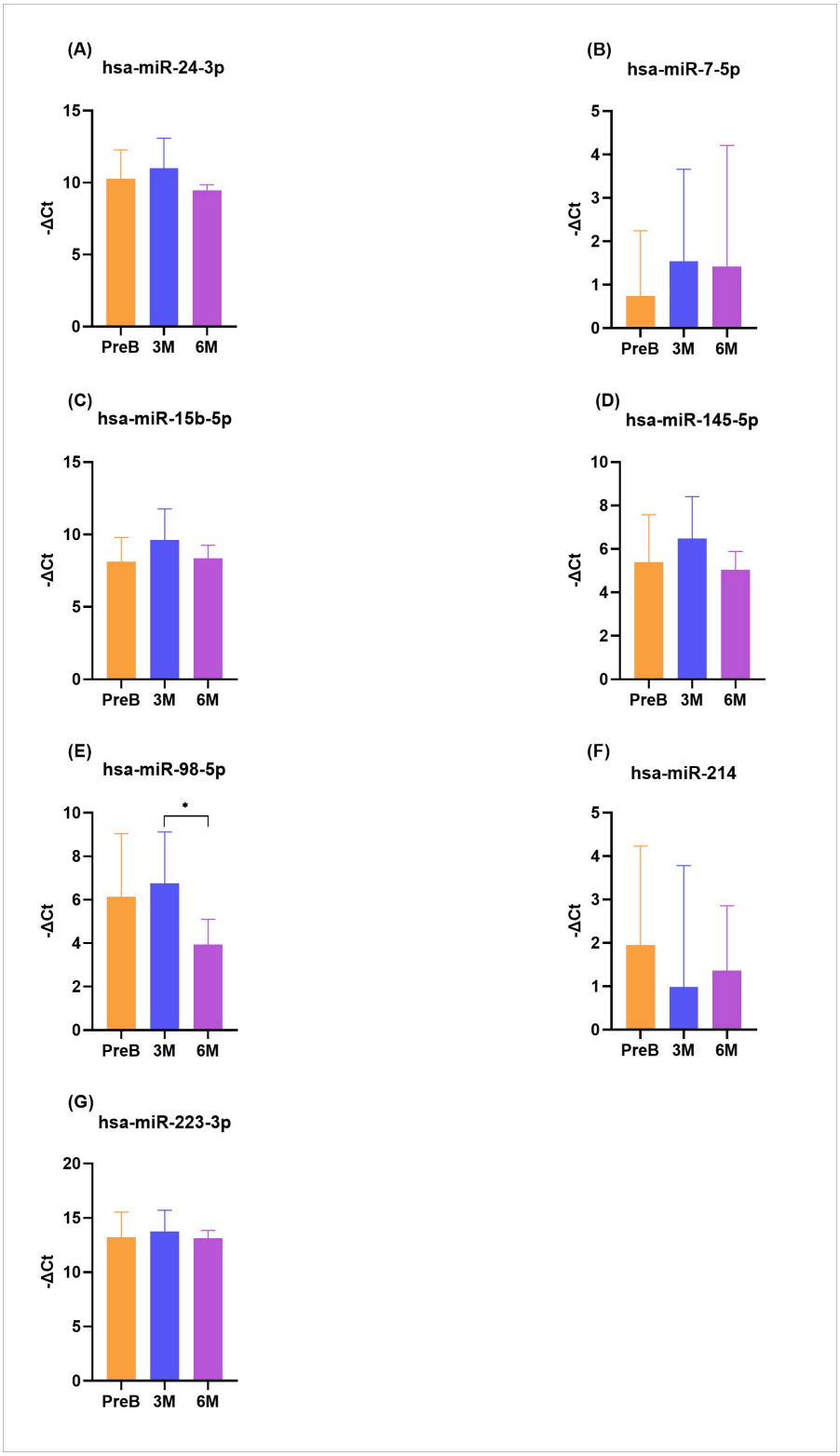


Figure 4

Quantification of miRNAs in hematologic tumors of cohort 1 in pre-boost (PreB), 3 months (3M) and 6 months (6M) after booster dose. (A) hsa-miR-24-3p plasmatic expression with mean ± SEM of PreB (N = 12): 10.27 ± 0.58; 3M (N = 11): 11.0 ± 0.63; 6M(N = 6): 9.46 ± 0.16; (B) hsa-miR-7-5p plasmatic expression with mean ± SEM of PreB (N = 12): 0.74 ± 0.43; 3 M (N = 11): 1.54 ± 0.64; 6 M (N = 6): 1.42 ± 1.14; (C) hsa-miR-15-5p plasmatic expression with mean ± SEM of PreB (N = 12): 8.12 ± 0.48; 3M(N = 11): 9.60 ± 0.65; 6M(N = 6): 8.37 ± 0.36; (D) hsa-miR-145-5p plasmatic expression with mean ± SEM of PreB (N = 12): 5.40 ± 0.63; 3 M (N = 11): 6.48 ± 0.58; 6 M (N = 6): 5.03 ± 0.35; (E) hsa-miR-98-5p plasmatic expression with mean ± SEM of PreB (N = 12): 6.13 ± 0.84; 3 M (N = 11): 6.75 ± 0.71; 6 M (N = 6): 3.93 ± 0.48; (F) hsa-miR-214 plasmatic expression with mean ± SEM of PreB (N = 12): 1.95 ± 0.66; 3 M (N = 11): 0.99 ± 0.84; 6 M (N = 6): 1.36 ± 0.61; (G) hsa-miR-223-3p plasmatic expression with mean ± SEM of PreB (N = 12): 13.22 ± 0.67; 3 M (N = 11): 13.78 ± 0.58; 6 M (N = 6): 13.12 ± 0.29. *p<0,05

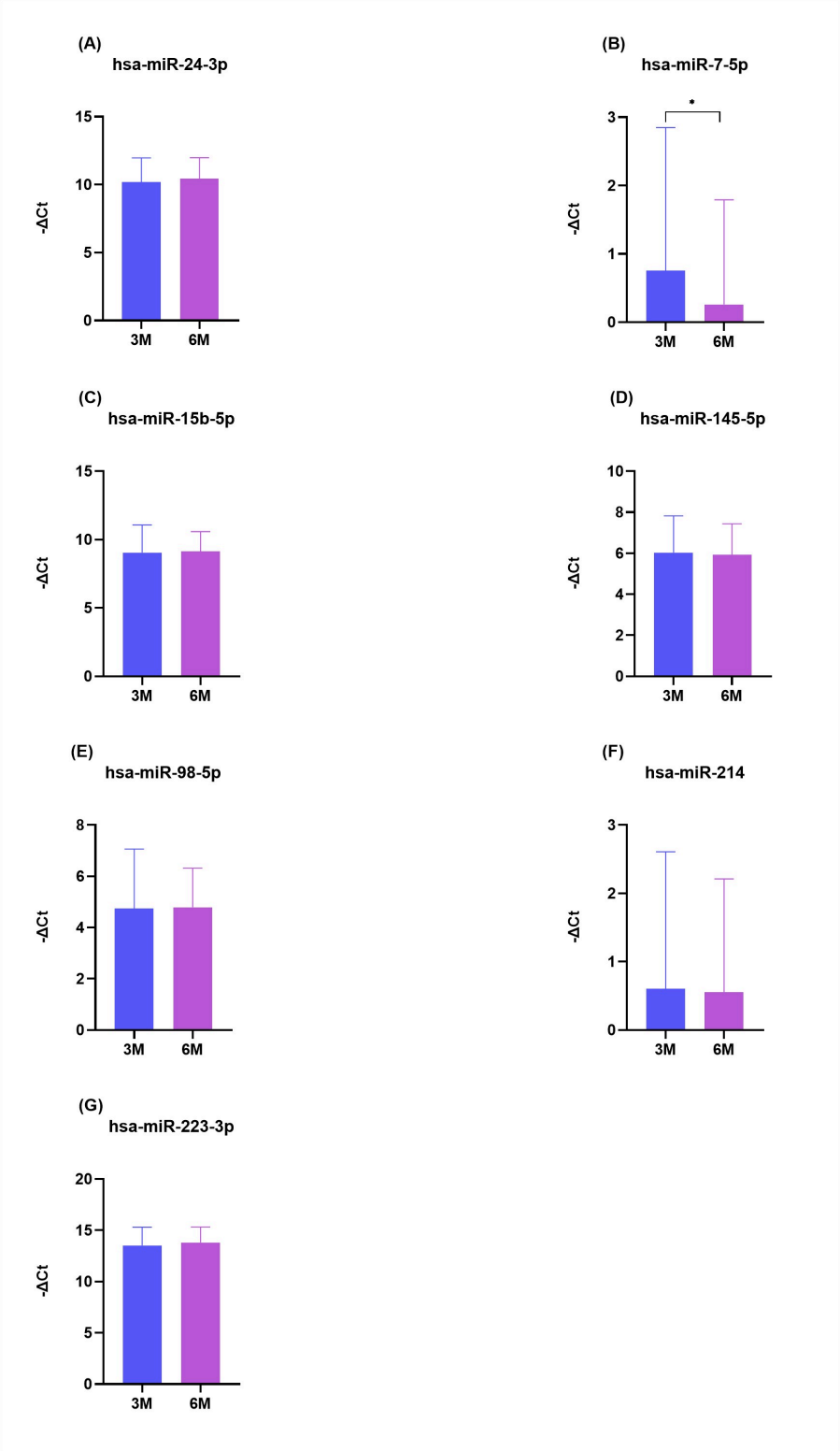


Figure 5

Quantification of miRNAs in solid cancer patients of cohort 1 and cohort 2, 3 months (3M) and 6 months (6M) after booster dose. A) hsa-miR-24-3p plasmatic expression with mean ± SEM of 3M (N = 221): 10.18 ± 0.12; 6M(N = 161): 10.44 ± 0.12; (B) hsa-miR-7-5p plasmatic expression with mean ± SEM of 3 M (N = 221): 0.75 ± 0.14; 6 M (N = 161): 0.26 ± 0.12; (C) hsa-miR-15-5p plasmatic expression with mean ± SEM of 3M(N = 221): 9.05 ± 0.14; 6M(N = 161): 9.13 ± 0.11; (D) hsa-miR-145-5p plasmatic expression with mean ± SEM of 3 M (N = 221): 6.03 ± 0.12; 6 M (N = 161): 5.92 ± 0.12; (E) hsa-miR-98 plasmatic

expression with mean ± SEM of 3 M (N = 221): 4.74 ± 0.16; 6 M (N = 161): 4.79 ± 0.12; (F) hsa-miR-214 plasmatic expression with mean ± SEM of 3 M (N = 221): 0.60 ± 1.14; 6M(N = 161): 0.55 ± 0.13; (G) hsa-miR-223-3p plasmatic expression with mean ± SEM of 3 M (N = 221): 13.52 ± 0.12; 6 M (N = 161): 13.82 ± 0.12. **p<0,01.

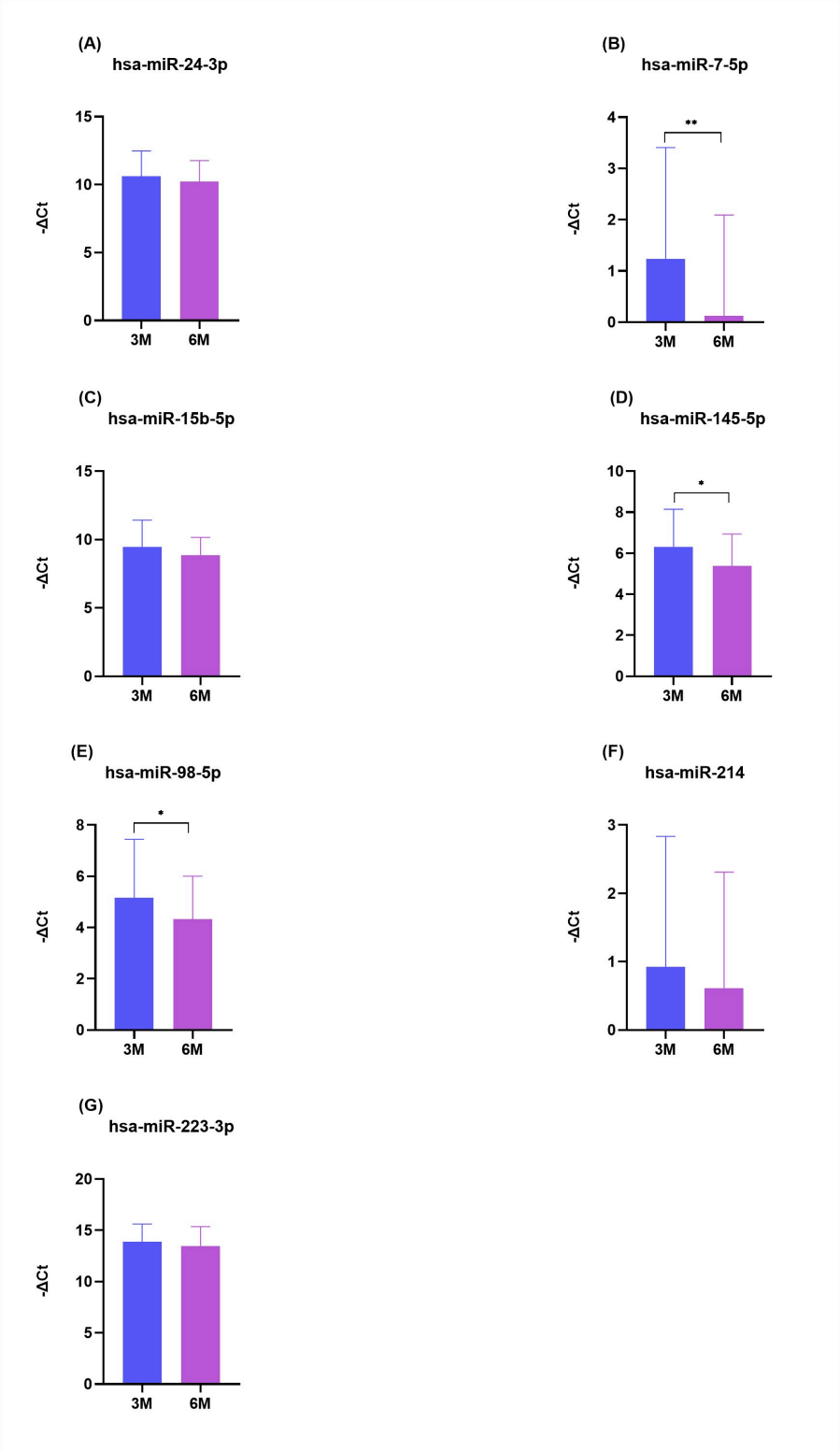


Figure 6

Quantification of miRNAs in hematological cancer patients of cohort 1 and cohort 2, 3 months (3M) and 6 months (6M) after booster dose. (A) hsa-miR-24-3p plasmatic expression with mean ± SEM of 3M (N =

39): 10.61 ± 0.30; 6M(N = 29): 10.21 ± 0.29; (B) hsa-miR-7-5p plasmatic expression with mean ± SEM of 3 M (N = 39): 1.24 ± 0.34; 6 M (N = 28): 0.12 ± 0.37; (C) hsa-miR-15-5p plasmatic expression with mean ± SEM of 3M(N = 39): 9.46 ± 0.31; 6M(N = 29): 8.87 ± 0.24; (D) hsa-miR-145-5p plasmatic expression with mean ± SEM of 3 M (N = 39): 6.32 ± 0.29; 6 M (N = 29): 5.39 ± 0.29; (E) hsa-miR-98-5p plasmatic expression with mean ± SEM of 3 M (N = 39): 5.16 ± 0.37; 6 M (N = 29): 4.33 ± 0.31; (F) hsa-miR-214 plasmatic expression with mean ± SEM of 3 M (N = 29): 0.92 ± 0.31; 6 M (N = 39): 0.61 ± 0.32; (G) hsa-miR-223-3p plasmatic expression with mean ± SEM of 3 M (N = 39): 13.87 ± 0.28; 6 M (N = 29): 13.48 ± 0.35. *p<0,05; ** p<0,01.

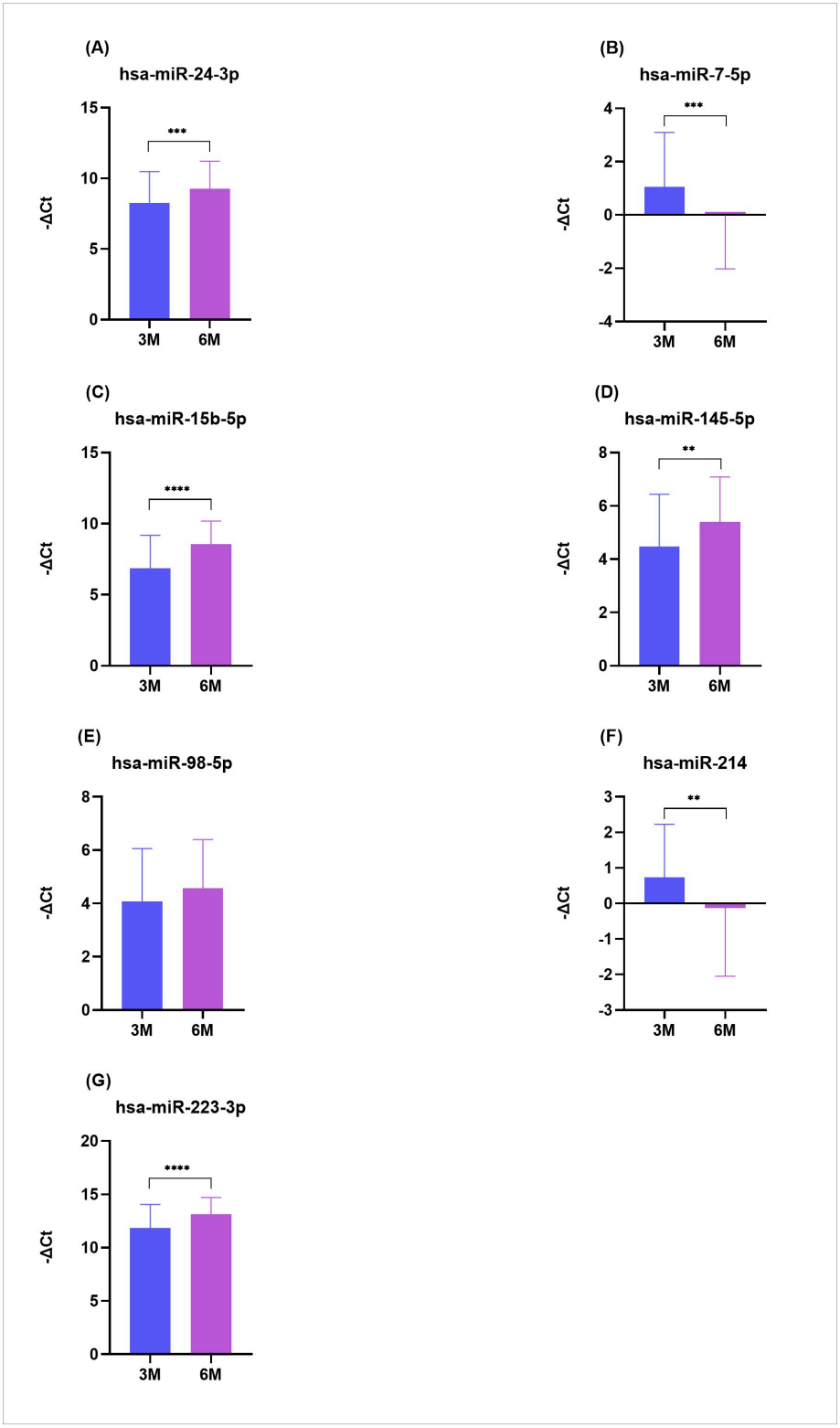


Figure 7

Quantification of miRNAs in healthy individuals, 3 months (3M) and 6 months (6M) after booster dose. A) hsa-miR-24-3p plasmatic expression with mean ± SEM of 3M (N = 82): 8.26 ± 0.24; 6M(N = 84): 9.27 ± 0.21; (B) hsa-miR-7-5p plasmatic expression with mean ± SEM of 3 M (N = 81): 1.06 ± 0.23; 6 M (N = 84): -0.01 ± 0.22; (C) hsa-miR-15-5p plasmatic expression with mean ± SEM of 3M(N = 82): 6.84 ± 0.26; 6M(N = 84): 8.55 ± 0.18; (D) hsa-miR-145-5p plasmatic expression with mean ± SEM of 3 M (N = 82): 4.49 ± 0.22; 6 M (N = 84): 5.40 ± 0.18; (E) hsa-miR-98-5p plasmatic expression with mean ± SEM of 3 M (N = 82): 4.08 ± 0.22; 6 M (N = 84): 4.58 ± 0.20; (F) hsa-miR-214 plasmatic expression with mean ± SEM of 3 M (N = 82): 0.73 ± 0.16; 6M(N = 84): -0.13 ± 0.21; (G) hsa-miR-223-3p plasmatic expression with mean ± SEM of 3 M (N = 82): 11.84 ± 0.25; 6 M (N = 84): 13.12 ± 0.17. **p<0,01; *** p<0,001; **** p<0,0001.

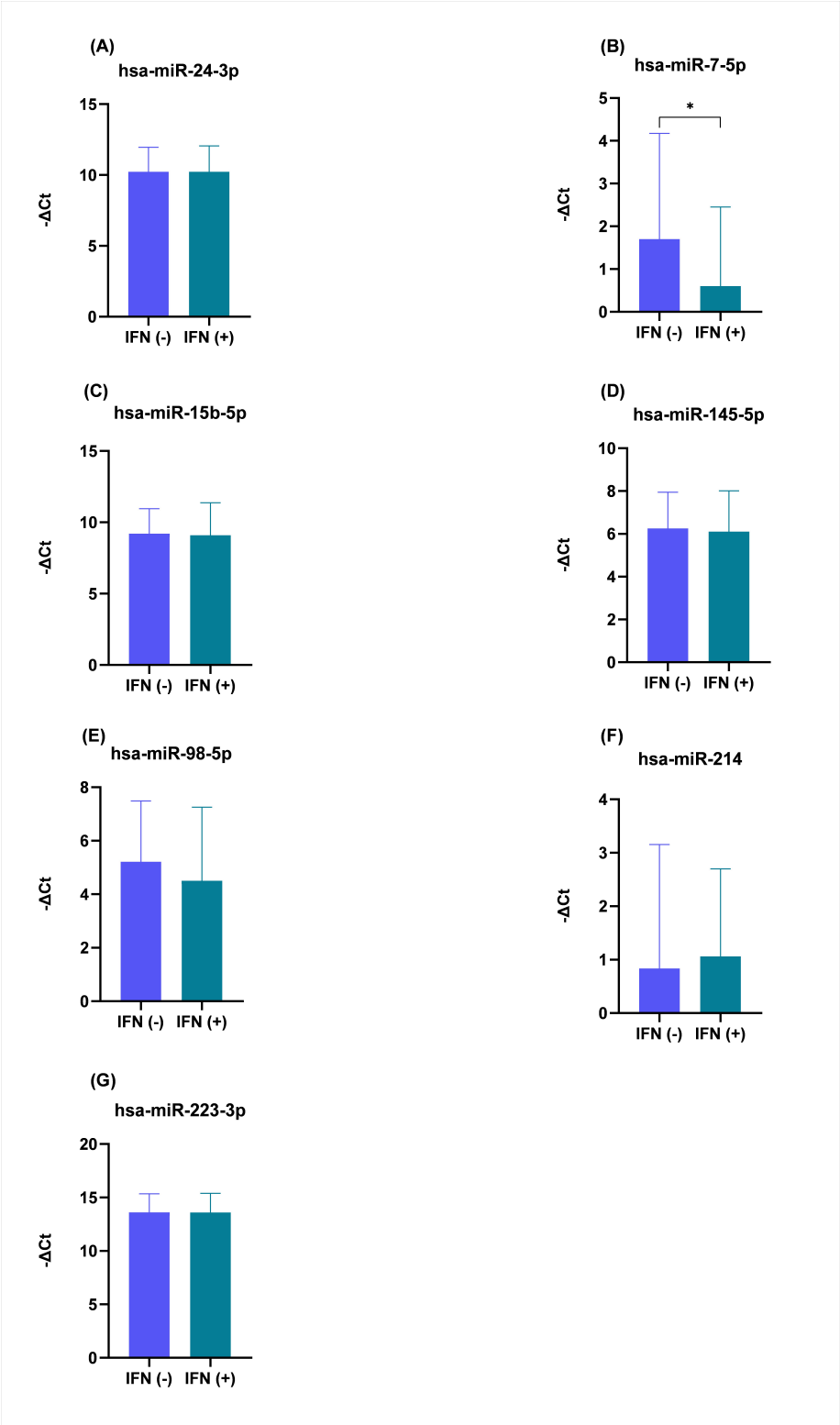


Figure 8

Quantification of miRNA expression in IFN (-) and IFN (+) patients. *p<0,05.