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Serum miR-338-3p and miR-199b-5p are associated with the absolute neutrophil count in patients with resectable pancreatic cancer

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ABSTRACT

Background: Several peripheral blood cell counts and immune ratios, such as the systemic immune-inflammation index (SII = platelet x neutrophil count/lymphocyte count) have prognostic value in patients with resectable pancreatic cancer (PDAC). Circulating microRNAs (miRNAs) are involved in various aspects of cancer and inflammation. We aimed to identify measurable circulating miRNAs in PDAC patients correlating with systemic inflammation parameters.

Methods: A total of 42 PDAC patients was included in this study: twelve in the discovery (n = 6 SII low; n = 6 SII high) and 30 patients in the validation cohort (n = 19 SII low, n = 11 SII high). MiRNAs isolated from preoperative serum samples were measured with a 352 miRNA panel in the discovery cohort and individual miRNA primers in the validation cohort, using RT-qPCR (ID3EAL assays, MiRXES).

Results: Only in the discovery cohort miR-328-3p, miR-338-3p, miR-1258 and miR-199b-5p were upregulated in high compared to low SII patients (fold difference ≥ 2, $P < 0.05$). In the total cohort (n = 42) correlations were found between miR-338-3p (r = 0.48, P = 0.002) and miR-199b-5p (r = 0.44, P = 0.005) and the absolute neutrophil count.

Conclusion: Circulating miR-338-3p and miR-199b-5p are correlated to the neutrophil count in the blood of PDAC patients, suggesting a potential role of circulating miRNAs in cancer immune evasion and systemic inflammation.

1. Introduction

Pancreatic cancer (PDAC) has a poor prognosis. Only 10% of patients presents with stage I resectable disease and although their prognosis is much better compared to patients with advanced disease, 5-year overall survival (OS) of stage I disease is only 34% [1]. Moreover, the mortality and morbidity of pancreatic tumor resection is high [2] and 80% of resected patients have disease recurrence within two years [3]. Patient stratification for therapy, such as surgical resection, is important to prevent patients from receiving inefficacious treatment and related complications.

Several studies have shown that immune cell counts, for example absolute neutrophil or lymphocyte counts, and immune cell ratios in the peripheral blood are of prognostic and predictive value in pancreatic cancer [4,5]. The number of neutrophilic granulocytes is associated with a shorter OS in patients with resectable disease and locally advanced PDAC [6]. Similarly, the neutrophil-to-lymphocyte ratio (NLR), the platelet-to-lymphocyte ratio (PLR), and the systemic immune-inflammation index (SII) are associated with poor prognosis in patients with resectable and non-resectable PDAC [7–10]. The SII was introduced by Hu et al. and is a ratio of the circulating platelet (P), neutrophil (N) and lymphocyte (L) counts (SII = P × N/L) [11]. The reference value of the SII is 459, based on a population-based prospective cohort study of individuals in the Netherlands aged 65.9 years on average [12]. The SII was found to be a prognostic marker for cancer-specific survival and disease recurrence in resectable PDAC and seems to outperform other immune ratios, such as the NLR [9,10]. A high SII (> 900) before resection is associated with worse prognosis compared to a low SII (≤900) before resection [9]. However, the underlying mechanism of an altered immune system reflected by an

Abbreviations: cDNA, complementary DNA; CSS, cancer-specific survival; Ct, threshold cycle; DE, differential expression; miRNA, microRNA; NLR, neutrophil-to-lymphocyte ratio; OS, overall survival; PDAC, pancreatic ductal adenocarcinoma; PFS, progression-free survival; PLR, platelet-to-lymphocyte ratio; qPCR, quantitative polymerase chain reaction; RT, reverse transcription; SII, systemic immune-inflammation index

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increased SII leading to poorer prognosis in PDAC remains unclear.

Cell-free circulating microRNAs (miRNAs) are small non-coding RNAs that regulate gene expression [13]. In the context of cancer, miRNAs play a role in several processes, such as apoptosis, cell proliferation, tumor suppression and immune response [13–15]. The majority of circulating miRNAs originates from endothelium and blood cells, including leukocytes and platelets [16]. Serum miRNAs mostly target immune related mRNAs [17]. Therefore, circulating miRNAs could reflect the status of the immune system.

In this study, we aim to identify differentially expressed miRNAs in the serum of resectable PDAC patients to explore the role of miRNAs in systemic inflammation.

2. Materials and methods

2.1. Patients and samples

Treatment-naïve patients with resectable PDAC were retrospectively identified based on availability of preoperative serum and absolute leukocyte and platelet counts. All patients underwent surgical resection of the primary tumor within the Erasmus University Medical Center Rotterdam in the period of February 2013 – December 2017.

Peripheral venous blood samples from PDAC patients were collected preoperatively on the day of surgery or up to five days prior to surgery. This study was approved by the Medical Ethics Committee of the Erasmus University Medical Center Rotterdam for which all patients had provided written informed consent (MEC-2015-085). Blood was collected in serum tubes with clot activator of silica particles (BD, Franklin Lakes, NJ). None of the patients showed signs of infection that might indicate, for example, cholangitis or pancreatitis at time of blood collection. After collection, blood samples were centrifuged for ten minutes at 2000 g, and serum was stored at −80 °C until further use.

Absolute circulating neutrophil, lymphocyte, and platelet counts were measured as part of standard preoperative patient care. SII values were calculated as the absolute platelet count, multiplied by the absolute neutrophil to absolute lymphocyte ratio (SII = P × N/L). Patients were grouped as low SII (median 592.0, interquartile range 451.6–720.3) and high SII (median 1227.7, interquartile range 1054.4–2016.4), based on the cutoff value of 900 determined by Aziz et al. SII values closest to the time of surgery were used for analysis. A total of 42 serum samples were available for this study. Twelve serum samples were used in the discovery cohort, six with the lowest SII and six with the highest SII, to select miRNAs for validation and correlation analyses. The remaining 30 serum samples, including 19 with a low SII and 11 with a high SII, were used in the validation cohort.

In this study, SII low patients were considered the control group. No other control samples were included, such as samples from chronic pancreatitis patients, because of the lack of evidence concerning the prognostic value of the SII in this patient group.

2.2. Serum miRNA isolation and quantitation

In the discovery cohort, RNA was isolated from 2 × 200 μL serum using the miRNeasy serum/plasma miRNA Isolation Kit (Qiagen, Hilden, Germany) in order to reach sufficient RNA volume for reliable measurement of 384 miRNA targets. In the validation cohort only 20 targets were measured and therefore RNA was isolated from 200 μL serum using the same isolation kit. In the validation cohort, two proprietary pre-mixed spike-in ~20 nucleotide control RNAs (MiRXES, Singapore) with sequences distinct from annotated mature human miRNAs (miRbase version 21) were added to the lysis buffer prior to the serum miRNA isolation according to manufacturer's instructions, in order to evaluate RNA isolation efficiency.

In the discovery cohort, serum miRNAs were reverse transcribed (RT) using ID3EAL miRNA-specific oligo's and RT spike-in RNA (MiRXES, Singapore) in a multiplex reaction per manufacturer's instruction. Complementary DNA (cDNA) was stored at −20 °C up to two weeks and thawed only once. cDNA was added to the ID3EAL miRNA qPCR Master Mix, containing buffer, polymerase and the passive reference dye ROX, and transferred to pre-loaded ID3EAL 384 Target Assay Panel plates (MiRXES, Singapore), including 352 individual quantitative polymerase chain reaction (qPCR) primers, 16 RT spike-ins and 16 inter-plate controls. PCR amplification was performed with the 7500 Fast Real-Time PCR system (Applied Biosystems, Foster City, CA, USA). Raw threshold cycle (Ct) values were determined using the 7500 Software (version 2.3; Applied Biosystems, Foster City, CA, USA).

In the validation cohort, selected serum miRNAs were measured with ID3EAL RT-qPCR after reverse transcription, using individual miRNA primers (MiRXES, Singapore). Raw threshold cycle (Ct) values were determined using the 7500 Software (version 2.3; Applied Biosystems, Foster City, CA, USA) and the serum miRNA samples were measured in duplicate.

2.3. Statistical analyses

Patient characteristics were compared using a Chi-square test for categorical and Mann-Whitney *U* test for numeric data. Progression-free survival (PFS) and cancer-specific survival (CSS) were calculated as the time between resection and radiologically confirmed progression or death caused by progression of the disease, respectively, comparing Kaplan-Meier curves with log-rank tests. Statistical analyses were performed with SPSS Statistics for Windows (version 24.0; IBM, Armonk, NY, USA) and Prism for Windows (version 5.01; GraphPad Software, San Diego, CA, USA).

In the discovery cohort, raw Ct values were normalized using reference miRNAs, spike-ins, and inter-plate calibrators, using an algorithm incorporated in the Cancer Panel Analysis Template (version 1.9, MiRXES, Singapore), provided by the manufacturer. The two miRNAs with the most stable expression among all samples were selected as reference miRNAs, using NormFinder software for Excel (version 0.953; MOMA, Aarhus University Hospital, Denmark). The cutoff values for detection of raw Ct values were set at a minimum of 9 and maximum of 33 cycles, based on the manufacturer's recommendation. The average miRNA expression of the low and high SII samples were compared with a two-tailed *t*-test.

Differentially expressed miRNAs between the high SII and low SII patients with a fold difference of ≤ 0.5 or ≥ 2, for downregulated or upregulated miRNAs respectively, and $P < 0.05$, that were detectable within the Ct value limits in all 12 patient samples, were selected for validation and continuous correlation analyses.

In the validation cohort, raw Ct values were normalized using the same two reference miRNAs from the discovery cohort. MiRNA expression relative to the reference miRNAs was calculated with the delta Ct method; expression $= 2^{-(\text{Ct miRNA of interest} - \text{average Ct reference miRNAs})}$. The average miRNA expression was compared between patient groups using a two-tailed *t*-test.

Correlations between miRNA expression and SII or individual absolute blood cell counts were measured by Pearson's correlation coefficient, using all patient samples, measured with individual qPCR. An univariate Cox regression analysis was conducted to evaluate the association between miRNA expression and CSS and PFS.

3. Results

3.1. Patient characteristics

A total of 42 treatment-naïve resectable PDAC patients were included in this study of whom serum was available and peripheral blood cell counts were measured before tumor resection at the Erasmus University Medical Center. No differences in patient characteristics were observed between the discovery cohort and validation cohort. In

Table 1
Patient characteristics.

	Discovery cohort (n = 12)	Validation cohort (n = 30)	P-value	SII low (n = 25)	SII high (n = 17)	P-value
Age (years), median (range)	70.0 (58–84)	69.5 (46–88)	0.466	68.0 (49–88)	71.0 (46–87)	0.635
Gender (%)						
Male	10 (83.3)	22 (73.3)	0.492	19 (76.0)	13 (76.5)	0.972
Female	2 (16.7)	8 (26.7)		6 (24.0)	4 (23.5)	
SII (%)						
Low (≤900)	6 (50.0)	19 (63.3)	0.426	25 (1 0 0)	0 (0)	NA
High (> 900)	6 (50.0)	11 (36.7)		0 (0)	17 (1 0 0)	
SII, median (IQR)	1139.8(398.5–2171.1)	744.0(592.5–1051.3)	1.000	592.0(451.6–720.3)	1227.7(1054.4–2016.4)	< 0.001
Absolute neutrophil count, median (IQR)	4.9 (3.4–8.1)	5.1 (4.4–5.6)	0.808	4.6 (3.6–5.3)	5.5 (4.8–7.0)	0.059
Absolute lymphocyte count, median (IQR)	1.3 (0.9–2.2)	1.7 (1.3–2.3)	0.353	2.2 (1.5–2.5)	1.1 (0.9–1.6)	0.012
Platelet count, median (IQR)	252.0(183.8–346.8)	291.0(238.0–326.0)	0.353	273.0(219.5–302.5)	303.0(240.5–380.0)	1.000
Bilirubin, median (IQR)	15.0 (7.5–54.3)	21.0 (9.5–29.5)	0.794	14.0 (8.5–28.5)	24.0 (9.5–51.5)	0.174
Tumor marker	200.0	47.0	0.258	40.0	82.0	0.530
CA19-9, median (IQR)	(25.6–348.0)	(20.5–299.0)		(20.0–305.5)	(38.5–334.5)	
Tumor location (%)						
Head	10 (83.3)	29 (96.7)	0.135	23 (92.0)	16 (94.1)	0.418
Body	0 (0)	1 (3.3)		1 (4.0)	0 (0)	
Tail	1 (8.3)	0 (0)		1 (4.0)	0 (0)	
Multifocal	1 (8.3)	0 (0)		0 (0)	1 (5.9)	
T-stage[a] (%)						
1	1 (8.3)	2 (6.7)	0.850	2 (8.0)	1 (5.9)	0.794
2	0 (0)	0 (0)		0 (0)	0 (0)	
3	11 (91.7)	28 (93.3)		23 (92.0)	16 (94.1)	
4	0 (0)	0 (0)		0 (0)	0 (0)	
N-stage[a] (%)						
0	4 (33.3)	7 (23.3)	0.505	6 (24.0)	5 (29.4)	0.695
1	8 (66.7)	23 (76.7)		19 (76.0)	12 (70.6)	
2	0 (0)	0 (0)		0 (0)	0 (0)	
Tumor differentiation (%)						
Good	0 (0)	0 (0)	0.395	0 (0)	0 (0)	0.497
Moderate	9 (75.0)	17 (56.7)		17 (68.0)	9 (52.9)	
Poor	3 (25.0)	10 (33.3)		6 (24.0)	7 (41.2)	
Unknown	0 (0)	3 (10.0)		2 (8.0)	1 (5.9)	
Adjuvant chemotherapy[b] (%)	6 (50.0)	10 (33.3)	0.315	14 (56.0)	12 (70.6)	0.339
CSS (months), median (95% CI)	22.0 (6.6–37.4)	18.0 (16.7–19.3)	0.666	20.0 (15.9–24.1)	16.0 (12.0–20.0)	0.484
PFS (months), median (95% CI)	20.0 (11.5–28.5)	13.0 (11.3–14.7)	0.457	14.0 (12.2–15.8)	13.0 (10.6–15.5)	0.455

SII = systemic immune-inflammation index; IQR = interquartile range; CSS = cancer-specific survival; CI = confidence interval; PFS = progression free survival.
[a] T- and N-stage classification according to AJCC 7th edition.
[b] Standard adjuvant chemotherapy consisted of six cycles of gemcitabine.

addition, no differences in patient characteristics were observed between SII low and SII high patients, except for the SII and absolute lymphocyte count, which was of course used to differentiate between patient groups. Patient characteristics are summarized in Table 1.

3.2. Differential serum miRNA expression in the discovery cohort

From the total cohort of PDAC patients, six patients with the highest SII values, and six patients with the lowest SII values were selected for serum miRNA discovery. Fig. 1 shows a scheme of the miRNA study design. A list of miRNAs included in the discovery ID3EAL Cancer miRNA Knowledge panel can be found in Supplementary Table 1. Out of 352 miRNAs, 207 were detectable within the Ct limits in all twelve serum samples. Four differentially expressed miRNAs (DE miRNAs) showed a fold difference ≥ 2 and $P < 0.05$ between the high SII and low SII group (Table 2). These four miRNAs were upregulated in the high SII group and were selected for validation in $n = 30$ additional patients. In the miRNA discovery samples we found that miR-345-5p and miR-130b-5p had the best stability and were selected as reference miRNAs for the validation cohort.

3.3. Serum miRNA expression in the validation cohort

In one out of 30 patient samples of the validation cohort we could not detect the four miRNAs of interest or the reference miRNAs by RT-qPCR and this sample was therefore excluded from further analysis. MiR-1258 was not detected in 10/60 meafvsurements (17%), in both the low SII as well as the high SII patients. None of the four selected miRNAs remained significantly upregulated between high SII and low SII patients in the independent validation set (Table 3). In contrast to our findings in the discovery cohort, miR-328-3p showed a significant downregulation in patients with high SII compared to low SII ($P = 0.022$).

3.4. Serum miRNA expression association with blood cell counts and survival

In addition to using an SII cutoff of 900 to separate the two patient groups, we calculated the correlations between expression data of miR-338-3p and miR-199b-5p and the SII and individual immune cell counts (Fig. 2). Two miRNAs were excluded from these analyses: miR-328-3p was excluded due to discrepancy in expression directionality between the discovery and validation cohort and miR-1258 was excluded due to the lack of detection.

There was a significant correlation between serum miR-338-3p (Fig. 2a) and the absolute neutrophil count (r = 0.48, $P = 0.002$) as well as between serum miR-199b-5p (Fig. 2b) and the absolute neutrophil count (r = 0.44, $P = 0.005$). Neither miR-338-3p or miR-199b-5p showed a significant correlation between serum expression and the SII ($P = 0.185$ and $P = 0.145$), the absolute lymphocyte count ($P = 0.453$ and $P = 0.280$) or absolute platelet count ($P = 0.576$ and $P = 0.918$).

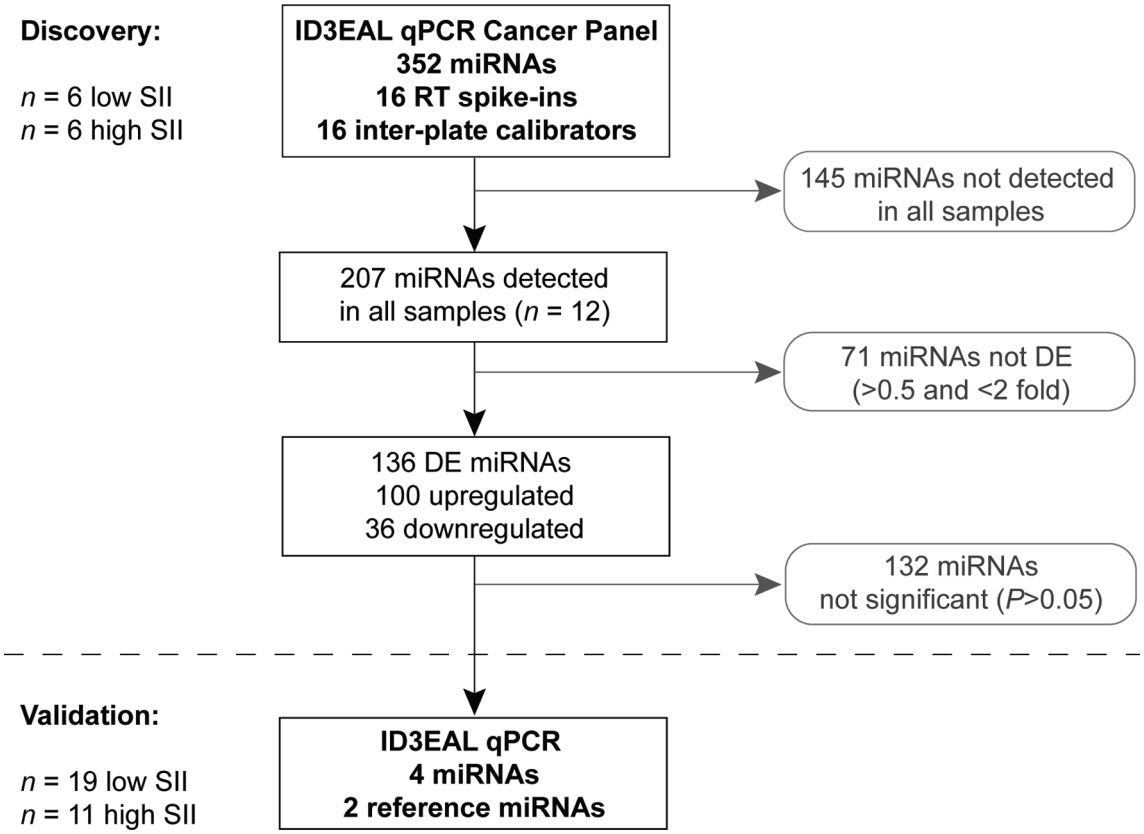

Fig. 1. Schematic overview of serum miRNA analysis. In the discovery cohort $n = 12$ patients with resectable pancreatic cancer with low or high systemic immune-inflammation index (SII) were analyzed using an RT-qPCR based 352 cancer miRNA panel from MiRXES to identify differentially expressed miRNAs (DE miRNAs). Statistical significance was calculated using a two-tailed *t*-test. Four DE miRNAs were measured in the validation cohort. RT = reverse transcription.

Table 2
Differentially expressed serum miRNAs in high SII versus low SII patients in the discovery cohort ($n = 12$) that were selected for validation.

MicroRNA	Fold difference	*P*-value
hsa-miR-328-3p	2.46	0.006
hsa-miR-338-3p	2.62	0.044
hsa-miR-1258	7.83	0.028
hsa-miR-199b-5p	2.01	0.022

Table 3
Differences in serum expression of the four selected miRNAs in high SII versus low SII patients of the validation cohort ($n = 30$).

MicroRNA	Fold difference	*P*-value
hsa-miR-328-3p	0.52	0.022
hsa-miR-338-3p	1.20	0.252
hsa-miR-1258	0.54	0.349
hsa-miR-199b-5p	1.12	0.662

To evaluate whether miR-338-3p and miR-199b-5p also show a correlation with survival, univariate Cox regression analyses were performed. There were no significant correlations between miR-338-3p and CSS (HR = 2.672, $P = 0.549$) or PFS (HR = 4.384, $P = 0.350$) or between miR-199b-5p and CSS (HR = 2.241, $P = 0.705$) or PFS (HR = 5.279, $P = 0.432$), as shown in Table 4.

4. Discussion

PDAC progression has been linked with the host's systemic inflammatory response and circulating blood cells [9,18]. This is the first study that attempts to identify a circulating miRNA signature related to the altered SII and immune cell counts in PDAC patients. MiRNAs are heavily involved in immune cell proliferation, differentiation and function [17,19,20]. Cell-free miRNAs in the circulation are primarily derived from blood cells and the endothelium [16]. Circulating miRNAs have potential as cancer biomarkers due to their stability in plasma or serum, their roles in cell-cell contact and regulation of cellular processes [21]. Although no correlation between the SII and any of the four miRNAs could be validated in our cohort, there was a statistically significant correlation between miR-338-3p and miR-199b-5p expression and the absolute neutrophil count. Whether miR-338-3p and miR-199b-5p are also upregulated in circulating neutrophils or tumor infiltrating neutrophils in PDAC patients needs to be investigated in the near future.

Neutrophilia is associated with a poor prognosis in PDAC [18,22] but the underlying mechanism is unknown. The chronic state of inflammation may well lead to an influx of immunosuppressive neutrophils into tumors [23] that recruit regulatory T cells in the tumors [24]. Immunosuppressive neutrophils generate high levels of reactive oxygen species (ROS) which blocks T cell proliferation [25] and can also induce CD8 T cell apoptosis through secretion of nitric oxide [26]. MiR-338-3p has not yet been described for its association with neutrophils or PDAC. However, miR-338-3p expression was found to be upregulated in leukocytes derived from patients with sporadic amyotrophic lateral sclerosis, showing that the disease related transcriptional miRNA alterations exist in tissues outside of the affected organ system [27]. Upregulation of miR-338-3p was also found in peripheral blood mononuclear cells of patients with pemphigus vulgaris, where it suppresses cell viability [28]. Whereas miR-338-3p is upregulated in the circulation in a limited number of studies, miR-338-3p is often downregulated in cancer tissues. Because of its tumor suppressing function,

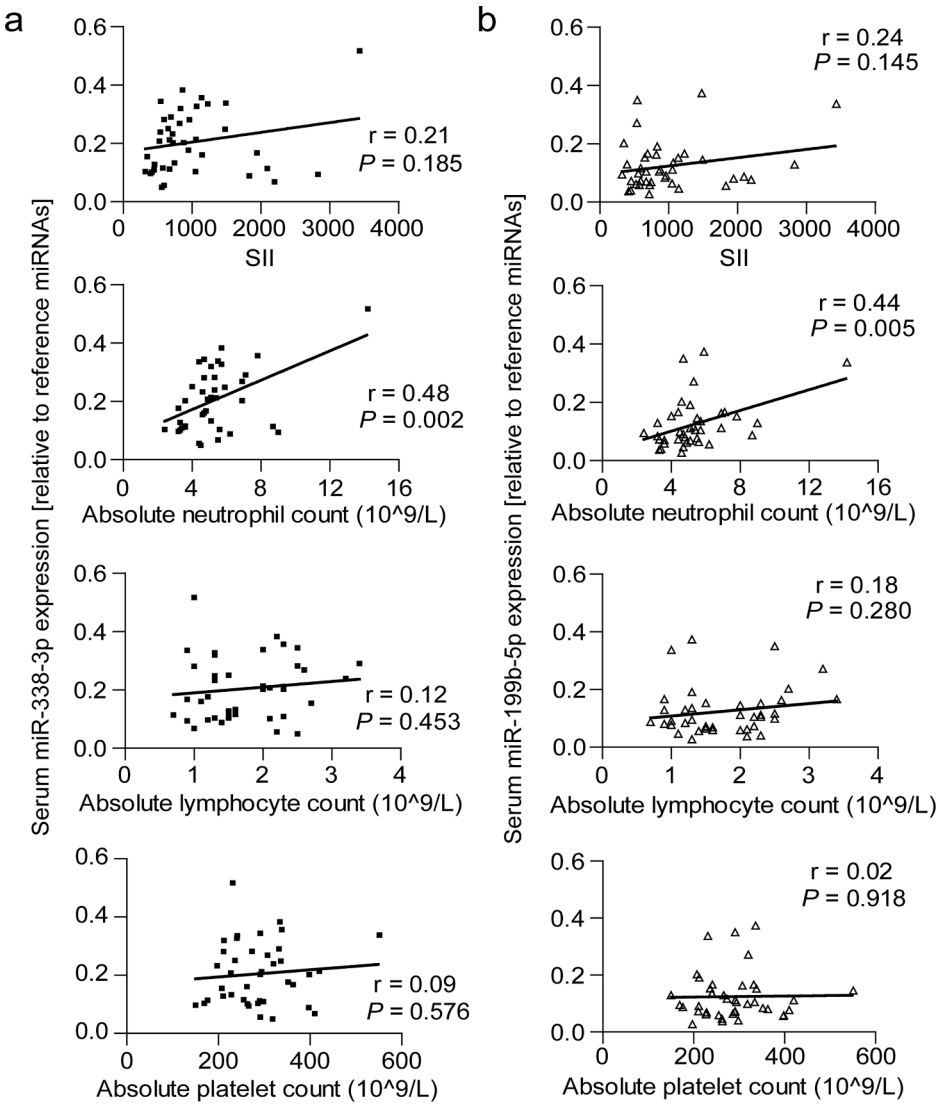

Fig. 2. Continuous relationship between serum miR-338-3p (a) and miR-199b-5p (b) expression and the systemic immune-inflammation index (SII) or individual absolute blood cell counts in the total cohort of patients (n = 42). Serum miRNA expression is relative to the average expression of reference miRNAs. r = correlation coefficient, P-value is calculated by Pearson's r correlation.

Table 4
Univariate Cox regression analysis of miR-338-3p and miR-199b-5p expression for cancer-specific survival (CSS) and progression-free survival (PFS) in patients with resectable pancreatic cancer.

MicroRNA	Hazard ratio* for CSS (95% CI)	P-value	Hazard ratio* for PFS (95% CI)	P-value
hsa-miR-338-3p	2.672 (0.107–66.579)	0.549	4.384 (0.198–97.144)	0.350
hsa-miR-199b-5p	2.241 (0.035–145.355)	0.705	5.279 (0.083–333.937)	0.432

* Hazard ratio for every 1 unit change, where each unit represents a change of 1.0 fold difference over reference miRNA expression. CI = confidence interval.

downregulation of miR-338-3p in cancer tissue samples was proven to be a poor prognostic factor in several cancers, such as gastric, and prostate cancer [29,30].

In a recent study miR-199b-5p expression in PDAC tissue was also linked with poor prognosis [31]. However, it remains unclear whether miR-199b-5p originates from the cancer cells themselves or from other cell types. Others have shown that the miR-199 family, including miR-199b, is primarily expressed in the fibrotic tumor associated stroma in PDAC tissues [32]. MiR-199b findings are inconsistent across studies in different cancers. For instance in cervical cancer, miR-199b-5p overexpression is associated with poor prognosis and metastasis [33], while in other cancers such as breast, and colorectal cancer miR-199b-5p has a tumor suppressor function and poor prognosis is reflected by a decrease of expression [34,35]. MiR-199b is overexpressed in myeloid progenitor cells and expression levels drop along with differentiation of these cells [36]. While in some studies miR-199b levels are reportedly upregulated in plasma of acute myeloid leukemia (AML) patients compared to healthy controls [37], in other studies high levels of miR-199b were found favorable in terms of OS of AML patients [36]. In sepsis patients, miR-199b-5p was specifically upregulated in blood cells (including neutrophils), but this difference in expression could not be detected in the serum [38]. Until now, the exact origin of disease-related circulating miRNAs remains unclear. Once in the circulation, miRNAs are transferred to various cell types where they can alter gene

expression depending on the cellular context and targeted organ [39,40].

MiRNA target prediction using the online database miRDB [41] showed that in general miR-338-3p has 601 predicted mRNA targets. The gene with the highest target score for miR-338-3p is Casitas B-lineage Lymphoma Proto-oncogene (CBL), which is a key player in balancing of the immune response and considered a potential target for anticancer immune therapy [42]. In neutrophils specifically, c-CBL mediates the termination of FcγRIIa signaling, resulting in down-regulation of neutrophil function [43]. For miR-199b-5p there are 556 predicted mRNA targets, from which Discoidin Domain Receptor Tyrosine Kinase 1 (DDR1) has the highest target score. DDR1 is known as a collagen receptor on leukocytes which plays a role in the physical attachment of leukocytes to collagen, as well as the differentiation and cytokine/chemokine production of cells of the myeloid lineage [44]. These findings suggest that miR-338-3p and miR-199b-5p could exert pivotal roles in the regulation of immune related gene expression.

The ID3EAL qPCR assay from MiRXES used in our study is a sensitive method for measuring serum miRNAs. This method utilizes miRNA-specific reverse transcription as well as a combination of a miRNA-specific forward and reverse primers. Two hundred and seven miRNAs included in the 352 miRNA panel were detected in all twelve discovery serum samples. This is higher than in qPCR microarrays without the use of miRNA-specific RT primers and is comparable to more advanced techniques, such as next generation sequencing and digital count technologies [45].

The main limitation of this retrospective study is the low number of patient samples. All patient samples had previously been collected from treatment-naïve patients, as part of our local blood sample biobank. Despite the large number of collected blood samples, only 42 serum samples were available for this study, due to missing data on absolute immune cells counts. For future studies, we are aiming to prospectively collect all data on, for example, immune cell counts, immune cell ratios and tumor markers in addition to blood samples for circulating biomarker analyses.

A significant prognostic value of the SII or circulating neutrophil counts could not be confirmed in this experiment. In addition, none of the investigated miRNAs showed an association between expression levels and survival, although there is a trend visible towards increased hazard ratios for CSS and PFS with increased expression of miR-338-3p and miR-199b-5p. The small sample size, causing wide confidence intervals, could explain the lack of statistical significance. Another unexpected finding is the discrepancy of miR-328-3p expression between the discovery and validation cohort, which might be explained by the high variability of miRNA expression in low SII patients. Although the SII cutoff value of 900 has been validated for its prognostic value in multiple studies, the SII might not be a good marker to differentiate between patient subgroups on a molecular level. However, the four miRNAs in the validation cohort of this study were selected based on the differential expression between low and high SII dichotomization. In retrospect, miRNA selection could have been improved by using, for example, the absolute neutrophil count to distinguish the two patient groups.

In conclusion, we aimed to identify serum miRNA signatures that are related to systemic inflammation in PDAC patients. Serum miR-338-3p and miR-199b-5p were found to be significantly correlated to the absolute neutrophil count in patients with resectable PDAC. Our findings support the potential role of miRNAs in the altered immune system of pancreatic cancer patients. Future research regarding circulating miRNAs in PDAC patients could elucidate whether miRNAs can serve as therapeutic target by altering immune related intercellular communication and reversing cancer immune evasion.

CRediT authorship contribution statement

Fleur van der Sijde: Conceptualization, Methodology, Validation, Formal analysis, Investigation, Writing - original draft, Visualization. **Eveline E. Vietsch:** Conceptualization, Methodology, Writing - review & editing, Supervision. **Dana A.M. Mustafa:** Resources, Writing - review & editing. **Yunlei Li:** Validation, Formal analysis. **Casper H.J. van Eijck:** Resources, Writing - review & editing, Supervision.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.cca.2020.03.005.

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