Assessment of miR-103a-3p in leukocytes—No diagnostic benefit in combination with the blood-based biomarkers mesothelin and calretinin for malignant pleural mesothelioma diagnosis

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Abstract

Malignant pleural mesothelioma (MPM) is a cancer associated with asbestos exposure and its diagnosis is challenging due to the moderate sensitivities of the available methods. In this regard, miR-103a-3p was considered to increase the sensitivity of established biomarkers to detect MPM. Its behavior and diagnostic value in the Mexican population has not been previously evaluated. In 108 confirmed MPM cases and 218 controls, almost all formerly exposed to asbestos, we quantified miR-103a-3p levels in leukocytes using quantitative Real-Time PCR, together with mesothelin and calretinin measured in plasma by ELISA. Sensitivity and specificity of miR-103a-3p alone and in combination with mesothelin and calretinin were determined. Bivariate analysis was performed using Mann-Whitney U test and Spearman correlation. Non-conditional logistic regression models were used to calculate the area under curve (AUC), sensitivity, and specificity for the combination of biomarkers. Mesothelin and calretinin levels were higher among cases, remaining as well among males and participants <60 years old (only mesothelin). Significant differences for miR-103a-3p were observed between male cases and controls, whereas significant differences between cases and controls for mesothelin and calretinin were determined. Positive correlations for miR-103a-3p were observed with age, environmental asbestos exposure, years with diabetes mellitus, and glucose levels, while negative correlations were observed with years of occupational asbestos exposure, creatinine, erythrocytes, direct bilirubin, and leukocytes. The addition of miR-103a-3p to mesothelin and calretinin did not
increase the diagnostic performance for MPM diagnosis. However, miR-103a-3p levels were correlated with several characteristics in the Mexican population.

Introduction

Malignant pleural mesothelioma (MPM) is a lethal cancer of the pleura caused by exposure to asbestos fibers, which is considered a group I carcinogen by the International Agency for Research on Cancer (IARC) and has been banned in more than 60 countries worldwide [1,2].

Three different MPM subtypes exist: epithelioid (the most frequent), sarcomatoid, and biphasic [3]. MPM shows a latency period of 20 to 50 years after asbestos exposure and is usually diagnosed at later stages of the disease, due to non-specific symptoms and moderate sensitivity of current diagnostic methods [3,4]. Moreover, response to current treatments is poor, and thus, survival is commonly low. Nonetheless, the combination of ipilimumab and nivolumab showed an increased survival of patients with diagnosed MPM [5]. Currently, diagnosis of MPM is based on histopathology and immunohistochemistry (IHC), despite the low sensitivity of these methods [3,4]. Therefore, it has been proposed that certain combinations of non-invasive biomarkers might improve MPM diagnosis [6–9]. Among those, mesothelin and calretinin showed promising results [6]. Mesothelin is a 41 KDa glycoprotein present in mesothelial cells derived from the MSLN gene, which also encodes megakaryocyte potentiating factor (MPF) [10]. Elevated plasma mesothelin levels have been reported among MPM cases in several studies, which placed this biomarker as the most prominent biomarker for MPM diagnosis [11]. However, mesothelin alone has a relatively low sensitivity [10,12–14]. On the other hand, its combination with calretinin, a 29 kDa calcium-binding protein that is also found in mesothelial cells and functions as a diagnostic biomarker of MPM, has already been evaluated [6,8,15]. In this sense, Jiménez-Ramírez et al. reported a sensitivity of 82.7% in men and 86.8% in women of a Mexican population when both molecules were used jointly for MPM diagnosis [6,8]. The combination of calretinin and mesothelin was additionally shown to be feasible for the early detection of mesothelioma using plasma samples of mesothelioma patients up to 15 months prior to MPM diagnosis [15]. Yet, there is still room for improvement by including additional biomarkers to this evaluated combination.

In this regard, microRNAs (miRNAs) have also gained interest in the diagnosis of several diseases, including MPM. MicroRNAs are non-coding RNA molecules of about 22 nucleotides (nt), which can be determined in the bloodstream (plasma, serum, and leukocytes). These miRNAs regulate several biological processes such as cell differentiation, proliferation, and apoptosis, through imperfect pairing with messenger RNA (mRNA), thus under- or over-expression can occur in different health or disease conditions [12,16,17]. Furthermore, miR-16, miR-132-3p, miR-103a-3p, miR-548a-3p, miR-20, miR-16, miR-12, miR-48, miR-548a-3p, miR-20, miR-486, miR-625-3p, miR-32-3p, miR-197-3p and miR-1281, have been proposed as likely non-invasive plasma biomarkers for MPM [18–24]. Mainly miR-103a-3p, which can be detected in the cellular fraction of blood, might be a promising candidate biomarker for MPM diagnosis due to its increased sensitivity for MPM diagnosis in combination with mesothelin [25]. Testing the effectiveness of these biomarkers is crucial for populations with current asbestos exposure—despite being proven as human carcinogen, such as in Mexico, where more than 500 MPM cases occur each year since 2010, and an underreporting of 71% has been estimated, causing an MPM epidemic [26,27]. Therefore, the aim of this study was to evaluate miR-103a-3p in leukocytes, together with mesothelin and calretinin in plasma, as an additional biomarker for MPM diagnosis. Likewise, the role of clinical and sociodemographic variables that could modify miR-103a-3p levels in a Mexican population was explored.
Methods

A total of 108 cases and 218 controls, matched by sex and age (± one year), were analyzed in this study, including 82 cases and 212 controls from a previous study by Jiménez-Ramírez et al. [6], and 32 new participants (26 cases and 6 controls). Cases were defined as patients who attended medical examinations at the Mexican Social Security Institute (IMSS) and the National Institute of Respiratory Diseases (INER) in Mexico City, who had a confirmed MPM diagnosis by IHC. Controls were recruited from the National System of IMSS beneficiaries (SINDO)–a registry for retirees, and the National Institute for the Elderly (INAPAM)–an elderly general population registry [9]. Each participant signed an informed consent form prior to recruitment in the study. Socio-demographic data, detailed history of asbestos exposure, biochemical parameters such as glucose, creatinine, cholesterol, triglycerides, and a complete blood count were included. This project was approved by IMSS’ National Scientific and Ethical Research Commission, with registration number R-2011-785-069, and by INER’s Science and Bioethics in Research Committee with the registration number C30-12.

Blood samples collection

Six milliliters (mL) of venous blood were obtained using EDTA tubes and centrifuged at 2,500 xg for 10 minutes, within 30 minutes after blood collection. Plasma and leukocyte fraction were separated and frozen immediately at -70˚C.

Aliquots of plasma and leukocytes were shipped to Germany under stringent frozen conditions for determination of calretinin and miR-103a-3p.

Determination of mesothelin and calretinin

Mesothelin and calretinin were measured in plasma using commercial enzyme-linked immunosorbent assays (ELISA) for mesothelin (DY3265, R&D Systems, Minneapolis, MN) and calretinin (DLD Diagnostika GmbH, Hamburg, Germany) according to manufacturer’s instructions. All samples were analyzed in duplicate, and a 5% coefficient of variation was allowed.

Determination of miR-103a-3p

RNA was isolated from 100 μl leukocytes using the NucleoSpin miRNA kit (Macherey-Nagel GmbH & Co KG, Düren, Germany) according to manufacturer’s instructions. Subsequently, miR-103a-3p was determined as described elsewhere with miR-125a as reference using quantitative Real-Time PCR (qRT-PCR) [19]. Most of the samples (N = 275) were analyzed in 2015 using the 7300 Real-Time PCR System (Thermo Fisher Scientific, Darmstadt, Germany), whereas 51 samples were analyzed in 2021 using the QuantStudio 7 Pro PCR system (Thermo Fisher Scientific, Darmstadt, Germany). Differences between cases and controls were similar in 2015 and 2021 (S1A Fig). Although group differences between years exist, all miR-103a-3p levels measured in 2021 were within the range of the measurements from 2015 (S1B Fig). Indeed, Matias-Garcia et al. reported that miR-103a-3p is not altered by long-term frozen storage or freeze-thaw cycles [28]. Thus, all miR-103a-3p values were integrated in subsequent analyses.

Statistical analysis

As previously reported [6], biomarker levels differ between males and females. Consequently, biomarker concentrations were analyzed separately according to sex. Biomarker concentrations were reported as medians and interquartile range (IQR). Mann-Whitney U, Chi-squared
or Fisher’s exact test were used to compare medians and proportions between groups, considering an alpha level of 0.05. Spearman’s rank correlation coefficients were reported between variables. Receiver operating characteristics (ROC) curves were constructed to calculate the area under the curve (AUC) for each biomarker, sensitivities were calculated at fixed specificities for each individual biomarker and in combination. Stata 14 SE (StataCorp LLC, TX, USA) and GraphPad Prism 6 (GraphPad Software, La Jolla, CA, USA) were used for analyses and graphical presentation of data.

**Results**

The main characteristics of the study group are depicted in Table 1. In summary, 90 MPM cases and 179 controls were men, and 18 cases and 39 controls were women. Median age of the subjects was 62 years in cases and controls. Epithelioid mesothelioma was the most common subtype accounting for 94.4% (N = 102) of all cases. For most cases (96.3%; N = 104) and controls (91.3%; N = 199), a former exposure to asbestos (occupational or environmental) was recorded.

**miR-103a-3p, mesothelin, and calretinin levels**

The three biomarkers were determined in a total of 326 samples. The median level of miR-103a-3p in cases was 217.52 and in controls 298.17 (Table 2). Marginally significant differences (p = 0.05) were observed between male cases and controls (217.52 vs. 298.17, respectively) but not within females (Fig 1). Among controls, statistically significant differences were observed between age groups (≤60 years: 203.15 vs. >60 years: 379.03) (Table 2), which remained for men and women (Fig 2).

Statistically significant differences were also found among male controls with environmental exposure to asbestos (without exposure: 202.25 vs. with exposure: 359.53), urea nitrogen levels and direct bilirubin (Table 2). Also, statistically significant differences were observed in male cases according to different erythrocytes levels (≤4.5 x10⁶/mm³: 293.80 vs >4.5 x 10⁶/mm³: 151.16). Moreover, miRNAs did not differ by drinking habits (non-drinker: 820.35 vs. drinker: 201.09; p = 0.053) and by blood pressure levels in controls (280.13 vs. 387.70 between blood pressure below/equal to and above 120/80 mmHg, respectively; p = 0.286). Finally, among female cases differences were found by glucose levels below and above 120 mg/dL (164.27 vs. 708.74) still not statistically significant (Table 2).

Median mesothelin levels were higher in cases compared to controls (2.34 and 0.55 nmol/L; S1 Table), which remained statistically significant after sex stratification–male cases 2.34 nmol/L and controls 0.56 nmol/L; female cases 2.28 nmol/L and controls: 0.53 nmol/L (Fig 1). Likewise, male controls >60 years presented higher mesothelin levels compared to those ≤60 years (0.62 vs. 0.48 nmol/L; p = 0.001) (S1 Table). Respecting calretinin, cases presented significantly higher levels compared to controls (1.52 vs. 0.13 ng/mL; p = 0.286). Noteworthy, calretinin levels were significantly higher between men and women only within controls (0.11 ng/mL vs. 0.27 ng/mL) (S1 Table).

**miR-103a-3p correlations with different variables**

Mir-103a-3p levels showed no linear correlation with mesothelin and calretinin concentrations (Fig 3). MiR-103a-3p was negatively correlated with age in male cases (Spearman -0.08; 95% CI -0.29–0.13) and positively correlated in male controls (Spearman 0.18; 95% CI 0.04–0.32). Likewise, positive correlations with environmental asbestos exposure were observed among females. Similarly, the correlations with diabetes mellitus duration among male
Table 1. Main characteristics of malignant pleural mesothelioma cases and healthy controls by gender in a Mexican population.

| Variables                                      | Total sample | Cases | Controls | Men | Controls | Women | Controls |
|------------------------------------------------|--------------|-------|----------|-----|----------|-------|----------|
| Total sample                                   | 108 (33.1)   | 90 (33.5) | 179 (66.5) | 18 (31.6) | 39 (68.4) |
| Age (years) [Median (IQR)]                     | 62 (55–71)   | 63 (55–71) | 60 (53–68) | 61 (53–68) |
| ≤60 years                                      | 48 (48.4)    | 39 (43.3) | 77 (43.0) | 9 (50.0) | 19 (48.7) |
| >60 years                                      | 60 (55.6)    | 51 (56.7) | 102 (57.0) | 9 (50.0) | 20 (51.3) |
| Histological subtypes                           |              |        |          |     |          |       |          |
| Epithelioid                                    | 102 (94.4)   | 84 (93.3) |          | 18 (100) |           |
| Biphasic                                       | 2 (1.9)      | 2 (2.2)  |          | -    |          | -     |          |
| Sarcomatoid                                    | 4 (3.7)      | 4 (4.5)  |          | -    |          | -     |          |
| Asbestos exposure No                            |              | 48 (44.4) |          | 2 (11.1) | 3 (7.7)  |
| Yes                                            | 104 (96.3)   | 88 (97.8) | 163 (91.1) | 16 (88.9) | 36 (92.3) |
| Occupational exposure No                       |              | 26 (24.1) |          | 13 (72.2) | 33 (84.6) |
| Yes                                            | 82 (75.9)    | 77 (85.6) | 122 (68.2) | 5 (27.8) | 6 (15.4)  |
| Years of occupational exposure [Median (IQR)]   | 11.5 (2–28)  | 12 (2–28) | 18 (5–29) | 10 (8–10) | 9.5 (3–24) |
| Environmental exposure No                      |              | 31 (28.7) |          | 3 (16.7) | 13 (33.3) |
| Yes                                            | 77 (71.3)    | 62 (68.9) | 119 (66.5) | 15 (83.3) | 26 (66.7) |
| Years of environmental exposure [Median (IQR)]  | 30 (18–42)   | 29.5 (17–49) | 32 (17–49) | 31 (30–42) | 37.5 (21–42) |
| Previous chemotherapy No                       |              | 82 (75.9) |          | 13 (72.2) |          |
| Yes                                            | 26 (24.1)    | 21 (23.3) |          | 5 (27.8) |          |
| Smoking status Non-smoker                      | 39 (36.1)    | 27 (30.0) | 63 (35.2) | 12 (66.7) | 26 (66.7) |
| Current/ever smoker                            | 69 (63.9)    | 63 (70.0) | 116 (64.8) | 6 (33.3) | 13 (33.3) |
| Drinking habit Non-drinker                     | 1110.2)      | 4 (4.4)  | 11 (6.1) | 7 (38.9) | 20 (51.3) |
| Current/ever                                   | 97 (88.9)    | 86 (95.6) | 168 (93.9) | 11 (61.1) | 19 (48.7) |
| Blood pressure ≤120/80 mmHg                    | 83 (76.9)    | 70 (77.8) | 126 (70.4) | 13 (72.2) | 26 (66.7) |
| >120/80 mmHg                                   | 25 (23.1)    | 20 (22.2) | 53 (29.6) | 5 (27.8) | 13 (33.3) |
| Glucose levels ≤120 mg/dL                      | 77 (79.4)    | 66 (80.5) | 129 (82.2) | 11 (73.3) | 24 (80.0) |
| >120 mg/dL                                     | 20 (20.6)    | 16 (19.5) | 28 (17.8) | 4 (26.7) | 6 (20.0)  |
| Years with diabetes mellitus [Median (IQR)]    | 10 (3–17)    | 10 (4–16) | 7.5 (3–12) | 4 (2–20) | 6.5 (4–14) |
| Ureic nitrogen <20 mg/dL                       | 74 (77.9)    | 63 (77.8) | 137 (86.7) | 11 (78.6) | 26 (86.7) |
| ≥20 mg/dL                                      | 21 (22.1)    | 18 (22.2) | 21 (13.3) | 3 (21.4) | 4 (13.3)  |
| Creatinine <1.25 mg/dL                         | 92 (94.9)    | 78 (94.0) | 146 (93.0) | 14 (100) | 30 (100)  |
| >1.25 mg/dL                                    | 5 (5.1)      | 5 (6.0)  | 11 (7.0) | 0 (0.0) | 0 (0.0)   |
| Total proteins <6 dL                           | 14 (21.2)    | 14 (24.6) | 0 (0.0) | 0 (0.0) | 0 (0.0)   |
| >6 g/dL                                        | 52 (78.8)    | 43 (75.4) | 154 (100) | 9 (100) | 30 (100)  |
| Total bilirubin <1.45 mg/dL                    | 66 (100)     | 55 (100) | 148 (95.5) | 11 (100) | 30 (100)  |
| >1.45 mg/dL                                    | 0 (0)        | 0 (0.0)  | 7 (4.5) | 0 (0.0) | 0 (0.0)   |
| Direct bilirubin ≤0.3 mg/dL                    | 56 (88.9)    | 48 (88.9) | 146 (94.2) | 8 (88.9) | 30 (100)  |
| >0.3 mg/dL                                     | 7 (11.1)     | 6 (11.1) | 9 (5.8) | 1 (11.1) | 0 (0.0)   |
| Cholesterol <200 mg/dL                         | 16 (84.2)    | 15 (88.2) | 80 (51.0) | 1 (50.0) | 15 (50.0) |
| >200 mg/dL                                     | 3 (15.8)     | 2 (11.8) | 77 (49.0) | 1 (50.0) | 15 (50.0) |
| Triglycerides ≤150 mg/dL                       | 28 (82.3)    | 23 (82.1) | 83 (52.9) | 5 (83.3) | 9 (31.0)  |
| >150 mg/dL                                     | 6 (17.7)     | 5 (17.9) | 74 (47.1) | 1 (16.7) | 20 (69.0) |
| Erythrocytes ≤4.5 x10⁶/mm³                      | 24 (72.9)    | 22 (30.1) | 3 (1.9) | 4 (30.8) | 2 (6.9)   |
| >4.5 x10⁶/mm³                                   | 62 (72.1)    | 51 (69.9) | 154 (98.1) | 9 (69.2) | 27 (93.1) |
| Platelets ≤150,000/mm³                         | 4 (4.1)      | 3 (3.8)  | 10 (6.4) | 1 (5.9) | 1 (3.5)   |
| >150,000/mm³                                   | 93 (95.9)    | 77 (96.3) | 147 (93.6) | 16 (94.1) | 28 (96.5) |
| Leukocytes ≤11,000/mm³                         | 85 (86.7)    | 70 (85.4) | 155 (98.1) | 15 (93.7) | 29 (100)  |

(Continued)
controls and blood glucose levels among female controls were moderately to strongly positive (Fig 3) (S2 Table).

On the other hand, years of occupational exposure presented a negative correlation within male controls, yet the correlation was weak ($\rho = -0.128$, $p = 0.029$). Moreover, leukocyte levels, creatinine levels among female controls, direct bilirubin levels and erythrocytes among males were negatively correlated with miR-103a-3p (Fig 3) (S2 Table).

Individual and combined sensitivity and specificity of miR-103a-3p, mesothelin, and calretinin

At a fixed specificity of 95.5% for men and 97.4% for women, miR-103a-3p in both males and females presented low AUCs [0.426 (95% CI 0.355–0.497) and 0.437 (95% CI 0.284–0.589), respectively], with a sensitivity of 4.4% in men and 0% in women. Among males, an AUC of 0.894 (95% CI 0.847–0.941) and a sensitivity of 72.2% at 95.5% specificity was reported for mesothelin, whereas for calretinin an AUC of 0.931 (95% CI 0.889–0.972) and a sensitivity of 80.9% at 95.5% specificity was observed. Regarding females, mesothelin had a better performance, with an AUC of 0.947 (95% CI: 0.870–1.024) and a sensitivity of 88.9% at 97.4% specificity, in contrast to calretinin [AUC = 0.829 (95% CI: 0.706–0.951), 61.1% sensitivity and 97.4% specificity]. When mesothelin and calretinin were combined, sensitivity reached 80.9% in men (95.5% specificity) and 83.3% in women (97.4% specificity). When miR-103a-3p was included together with both biomarkers, there was no increase in sensitivity (Fig 4 and Table 3). Regarding age-related differences of miR-103a-3p levels, additional analyses were conducted in males $>$60 years (51 cases and 102 controls) and $\leq$60 years (39 cases and 77 controls), revealing improved performance of miR-103a-3p when stratified by age in terms of a larger area under the curve in the group of older men (0.6584 vs. 0.5361–among participants $>$60 years miR-103a-3p cutoff $\leq$39.671 resulted in a sensitivity of 9.8% and a specificity of 95.1%, whereas among participants aged $\leq$60 years, with miR-103a-3p cutoff $>$1438.152 resulted in a sensitivity of 12.8% and a specificity of 94.8% (S3 Table). Despite a doubling of the sensitivity of miR-103-3p as an individual marker in the subpopulation, the addition of miR-103a-3p to the marker combination of mesothelin and calretinin did not lead to an improved performance.

Discussion

Several studies in different populations have analyzed the combination of certain biomarkers at different molecular levels to improve MPM diagnosis [6–8,20,25,29]. In this regard, our study aimed to assess miR-103a-3p in leukocytes in addition to mesothelin and calretinin in

| Variables | Total sample | Men | Women |
|------------|--------------|-----|-------|
|            | Cases N (%)  | Controls N (%) | Cases N (%) | Controls N (%) | Cases N (%) | Controls N (%) |
| $>$11,000/mm$^3$ | 13 (13.3) | 3 (1.6)** | 12 (14.8) | 3 (1.9)** | 1 (6.3) | 0 (0.0) |

IQR, interquartile range.

Chi square test ($p < 0.05$).

Fisher’s exact test ($p < 0.05$).

Erythrocytes value considered in women was 4.2 x10$^6$/mm$^3$.
Table 2. Distribution of miR-103a-3p levels in leukocytes among malignant pleural mesothelioma cases and population controls according to sex groups in a Mexican population.

| Variables                | Total sample | Men | Controls | Women |
|--------------------------|--------------|-----|----------|-------|
|                          | Cases Median (IQR) | Controls Median (IQR) | Cases Median (IQR) | Controls Median (IQR) | Cases Median (IQR) | Controls Median (IQR) |
| miR-103a-3p              | 217.52 (94.03–466.33) | 298.17 (120.25–855.13) | 217.52 (95.00–474.41) | 298.17 (121.93–855.13) | 226.79 (93.05–404.50) | 248.38 (106.89–872.44) |
| Subtypes                 |              |     |          |       |     |                 |
| Epithelioid              | 213.80 (90.50–474.41) | - | 213.83 (89.57–485.81) | - | 226.79 (93.05–404.50) | - |
| Biphasic                 | 413.10 (121.93–704.27) | - | 413.10 (121.93–704.27) | - | - | - |
| Sarcomatoid              | 225.55 (162.08–234.92) | - | 225.55 (162.08–234.92) | - | - | - |
| Age                      |              |     |          |       |     |                 |
| ≤60 years                | 224.42 (103.71–668.60) | 203.15 (87.24–666.28) | 230.58 (108.38–704.27) | 229.12 (93.05–663.98) | 194.01 (93.05–326.28) | 166.57 (35.50–407.31) |
| >60 years                | 213.80 (89.57–381.71) | 379.03 (158.16–910.17) | 210.83 (88.64–377.41) | 379.03 (173.64–910.90) | 259.57 (137.74–959.34) | 423.64 (142.03–1121.23) |
| Occupational exposure    |              |     |          |       |     |                 |
| No                       | 292.28 (124.49–809.00) | 384.13 (140.06–872.44) | 304.43 (191.34–1060.11) | 418.76 (194.01–868.4) | 278.20 (124.49–596.34) | 315.17 (117.78–872.44) |
| Yes                      | 188.06 (90.50–386.02) | 254.25 (101.26–786.95) | 190.01 (90.50–410.14) | 262.32 (106.89–797.86) | 137.74 (93.05–164.27) | 141.55 (65.34–349.70) |
| p = 0.082                |              |     |          |       |     |                 |
| Environmental exposure   |              |     |          |       |     |                 |
| No                       | 219.79 (87.42–410.14) | 202.25 (87.24–666.28) | 202.95 (108.38–704.27) | 229.12 (74.02–689.78) | 194.01 (93.05–326.28) | 166.57 (35.50–407.31) |
| Yes                      | 216.76 (108.38–474.41) | 359.53 (158.16–910.17) | 217.52 (108.38–526.39) | 398.93 (173.64–910.17) | 259.57 (137.74–959.34) | 423.64 (142.03–1121.23) |
| p = 0.001                |              |     |          |       |     |                 |
| Previous Chemotherapy    |              |     |          |       |     |                 |
| No                       | 219.03 (88.64–458.25) | - | 218.27 (88.64–458.25) | - | 259.57 (124.49–404.50) | - |
| Yes                      | 157.72 (106.15–526.39) | - | 151.16 (108.38–526.39) | - | 164.27 (93.05–326.28) | - |
| Smoking status           |              |     |          |       |     |                 |
| Non-smoker               | 177.29 (80.44–386.02) | 298.17 (121.93–689.78) | 217.29 (80.44–386.02) | 298.17 (121.93–689.78) | 221.24 (101.64–461.31) | 271.35 (117.78–872.44) |
| Current/ever smoker      | 219.79 (108.38–474.41) | 308.68 (119.42–897.64) | 219.79 (108.38–497.22) | 308.68 (119.42–897.64) | 226.79 (93.05–404.50) | 243.38 (106.89–407.31) |
| Drinking habit           |              |     |          |       |     |                 |
| Non-drinker              | 278.20 (85.03–809.00) | 359.53 (135.29–995.99) | 820.35 (275.36–1609.58) | 600.49 (76.00–1074.91) | 164.27 (78.79–596.34) | 332.43 (137.68–934.21) |
| Current/ever             | 210.83 (95.00–410.14) | 280.13 (119.42–797.86) | 201.09 (90.50–458.25) | 292.06 (122.36–805.14) | 259.57 (124.49–404.50) | 227.54 (106.09–621.66) |
| Blood pressure           |              |     |          |       |     |                 |
| ≤120/80 mmHg             | 194.01 (90.50–404.50) | 280.13 (118.60–773.36) | 217.52 (90.50–497.22) | 284.07 (119.42–770.68) | 164.17 (93.05–304.43) | 243.61 (117.78–1082.38) |

(Continued)
Table 2. (Continued)

| Variables | Total sample | Cases | Controls | Men | Women |
|-----------|--------------|-------|----------|-----|-------|
|           | Median (IQR) | Median (IQR) | Median (IQR) | Median (IQR) | Median (IQR) |
| >120/80 mmHg | 259.57 (109.89–596.34) | 387.70 (135.29–922.88) | 217.98 (108.02–434.19) | 418.76 (161.60–1009.90) | 596.34 (259.57–809.00) | 315.17 (106.09–621.66) |
| Glucose levels | | | | | | |
| <120 mg/dL | 218.27 (88.64–377.41) | 394.34 (166.57–995.99) | 222.88 (88.64–526.39) | 393.44 (162.01–922.88) | 164.27 (85.03–278.20) | 383.42 (170.71–1121.2) |
| >120 mg/dL | 252.63 (104.47–708.74) | 399.70 (188.70–739.29) | 205.23 (104.47–398.08) | 472.58 (198.92–775.85) | 708.74 (344.69–832.25) | 137.20 (31.34–621.666) |
| Ureic nitrogen | | | | | | |
| <20 mg/dL | 206.14 (90.50–386.02) | 436.54 (177.29–1038.29) | 218.27 (90.50–474.41) | 486.86 (188.70–989.11) | 194.01 (85.03–326.28) | 383.42 (140.06–1160.07) |
| ≥20 mg/dL | 280.13 (185.33–704.27) | 221.07 (106.89–380.65) | 299.45 (185.33–704.27) | 221.07 (94.35–380.65) | 259.57 (93.05–843.35) | 242.50 (121.09–672.85) |
| Creatinine | | | | | | |
| <1.25 mg/dL | 214.55 (94.03–442.26) | 396.18 (170.10–995.99) | 219.03 (95.00–497.22) | 403.12 (184.82–989.11) | 179.14 (93.05–304.43) | 359.53 (139.10–1082.38) |
| ≥1.25 mg/dL | 280.13 (109.89–324.43) | 229.12 (133.43–486.86) | 280.13 (109.89–324.43) | 229.12 (133.43–486.86) | - | - |
| Total proteins | | | | | | |
| <6 g/dL | 215.31 (88.64–301.99) | - | 215.31 (88.64–301.99) | - | - | - |
| ≥6 g/dL | 279.17 (141.87–746.03) | 390.52 (164.29–955.99) | 280.13 (146.01–955.42) | 396.18 (177.29–916.07) | 259.57 (173.74–304.43) | 359.53 (139.10–1082.38) |
| Total bilirubin | | | | | | |
| <1.5 mg/dL | 245.57 (109.89–666.28) | 390.52 (166.57–922.88) | 247.27 (109.89–704.27) | 396.18 (181.05–913.12) | 194.01 (93.05–304.43) | 359.53 (139.10–1082.38) |
| ≥1.5 mg/dL | - | 242.19 (87.24–1038.29) | - | 242.19 (87.24–1038.29) | - | - |
| Direct bilirubin | | | | | | |
| <0.3 mg/dL | 265.08 (128.58–685.28) | 403.12 (170.10–995.99) | 275.36 (132.72–620.02) | 411.59 (184.82–989.11) | 226.79 (108.26–291.32) | 359.53 (139.10–1082.38) |
| ≥0.3 mg/dL | 190.01 (88.03–670.92) | 187.40 (87.24–364.55) | 149.95 (88.03–670.92) | 187.40 (87.24–364.55) | 596.34 | - |
| Cholesterol | | | | | | |
| <200 mg/dL | 320.30 (173.65–668.60) | 418.76 (191.34–872.44) | 354.58 (280.13–670.92) | 413.04 (191.34–846.33) | 41.64 | 436.54 (166.57–1082.38) |
| ≥200 mg/dL | 191.34 (39.67–821.15) | 357.06 (131.20–1024.09) | 115.50 (39.67–191.34) | 377.41 (151.16–1009.90) | 821.15 | 349.70 (84.44–1160.07) |
| Triglycerides | | | | | | |
| ≤5.4x109/mm³ | 282.87 (194.01–526.39) | 162.01 (87.24–359.53) | 293.80 (225.97–526.39) | 162.01 (87.24–296.11) | 179.14 (102.96–507.58) | 249.80 (112.25–1721.14) |
| >5.4x10⁹/mm³ | 190.01 (88.34–390.95) | 407.31 (173.64–995.99) | 151.16 (84.44–377.41) | 411.59 (177.29–922.88) | 304.43 (259.57–404.50) | 407.31 (166.57–1082.38) |
| Erythrocytes | | | | | | |
| ≤4.5x10⁹/mm³ | | | | | | |
| >4.5x10⁹/mm³ | | | | | | |
| Platelets | | | | | | |

(Continued)
However, the addition of this third miRNA marker did not increase the performance of mesothelin and calretinin for MPM diagnosis in a Mexican population.

Initially, miR-103a-3p was described as a biomarker for mesothelioma using the cellular fraction of blood [19]. A further study by Weber et al. confirmed that miR-103a-3p was lower in individuals with MPM in comparison to asbestos-exposed controls and controls from the general population, but there were no differences according to histological subtypes [25]. In our study miR-103a-3p was also detectable in leukocytes and lower levels were observed in MPM cases compared to controls. However, differences were present only among males, whereas among females no differences in miR-103a-3p were observed between cases and controls. In contrast, Weber et al. reported no differences in miR-103a-3p levels between men and women, regardless of case and control status, probably due to small sample size—five female cases and one asbestos-exposed control [19]. Hence, more research efforts in bigger study groups are needed to evaluate the expression of miR-103a-3p in women. Regarding smoking status, our results for miR-103a-3p were consistent with those reported by Weber et al. with no differences between smokers and non-smokers [19]. We also observed a higher expression of miR-103a-3p among controls aged >60 years, which could be explained by the presence of non-communicable diseases such as high blood pressure, diabetes mellitus, osteoporosis, arthritis, decreased kidney function, and cardiovascular diseases, which cause certain miRNAs to be over- or under-expressed [30]. Nonetheless, this overexpression among males >60 years did not improve the diagnostic performance of the combination mesothelin plus calretinin. Particularly, high miR-103a-3p plasma levels have been reported among individuals with high blood pressure and kidney injury [31,32]. This might be relevant because reduced renal function can be an influencing factor of circulating biomarkers as has been shown for calretinin and mesothelin [33]. In our study, only one individual presented with chronic kidney disease and unfortunately no markers of early renal damage were determined to evaluate the behavior of miR-103a-3p in this clinical condition. In the case of high blood pressure, we did not find differences in miR-103a-3p expression, possibly because this information was obtained by questionnaire and not by measurement. The differences found in male controls in relation to urea nitrogen levels in our study could support this issue. However, in order to clarify the mechanisms behind kidney function and miRNA expression, it is necessary to evaluate miR-103a-3p in different metabolic conditions. For instance, in our study these conditions were only evaluated by questionnaire or determined indirectly by biochemical parameters such as

| Variables | Total sample | Men | Women |
|-----------|-------------|-----|-------|
|           | Cases Median (IQR) | Controls Median (IQR) | Cases Median (IQR) | Controls Median (IQR) | Cases Median (IQR) | Controls Median (IQR) |
| ≤150000/ mm³ | 544.57 (235.07–1227.55) | 491.14 (184.82–2105.57) | 280.13 (190.01–1646.10) | 434.27 (184.82–1715.60) | 809.00 | 3082.74 |
| >150000/ mm³ | 218.27 (99.04–410.14) | 380.65 (161.60–922.88) | 218.27 (97904–474.41) | 387.61 (162.01–916.07) | 226.79 (1087.73–365.39) | 359.53 (139.58–1039.19) |
| Leukocytes | ≤11000/mm³ | 210.83 (90.50–497.22) | 393.27 (170.10–992.55) | 213.80 (88.64–526.39) | 398.93 (177.29–922.88) | 194.01 (93.05–404.50) | 359.53 (140.06–1082.38) |
| >11000/mm³ | 218.27 (185.33–280.13) | 119.42 (8.39–812.42) | 204.80 (146.85–299.45) | 119.42 (8.39–812.42) | 259.57 | - |

IQR, Interquartile range.
All comparisons were tested with Mann-Whitney U test.

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glucose, blood ureic nitrogen, and creatinine. Likewise, it was not possible to compare our results by histological subtype, since the number of cases with biphasic and sarcomatoid subtypes was too small.

Although miR-103a-3p has been reported to be upregulated in newly diagnosed diabetes mellitus (less than 5 years old), high blood pressure and kidney damage [31,32,34], in our study no significant changes in the expression of this miRNA were observed in relation to glucose levels >120 mg/dL. However, there were positive correlations in male controls with respect to years of diabetes mellitus and with glucose levels in female cases. It was previously reported that the miR-103a family could function as a biomarker of diabetes [35]. Considering that the prevalence of diabetes mellitus in Mexico in the population aged 60–69 years is 25.8%, and that in 2020 it was ranked third among all causes of mortality, its application could be explored as a marker for early diagnosis or surveillance in diabetic people in the Mexican population [36]. The negative correlation of miR-103a-3p with creatinine levels in the control group of women could suggest an involvement in kidney damage, as previously reported by

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**Fig 1.** Distribution of medians of mesothelin (A), calretinin (B) and miR-103a-3p (C) in malignant pleural mesothelioma cases and population controls in a Mexican population. Black bars represent cases and gray bars represent controls.

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Lu et al [37]. Remarkably, a positive correlation was found with environmental exposure for female controls but negative for males with occupational asbestos exposure. This might suggest that miR-103a-3p could possibly be useful as a marker of asbestos exposure rather than an MPM hallmark. Another finding in our study was that miR-103a-3p was positively correlated with age and was significantly different between participants \( \geq 60 \) years and \( < 60 \) years within the control group, similar to previously reported results [38].

Although it has been reported that miR-103a-3p can discriminate between MPM patients and people exposed to asbestos using extracellular plasma vesicles, when exploring its use as a prognostic biomarker in patients with MPM and after chemotherapy in the same biological matrix, the results have been conflicting [39,40]. Therefore, there is a need for further research to clarify the role of miR-103a-3p as a prognostic biomarker in patients with MPM. On the other hand, in other types of cancer such as colon cancer, breast cancer, and prostate cancer, miR-103a-3p has been considered a good candidate biomarker for diagnostics. In case of breast cancer, miR-103a-3p has shown to be upregulated in patients with breast tumors and after surgery the expression levels of this miRNA decreases, suggesting a potential role as a marker for treatment follow-up [41–43]. Within some other diseases such as endometriosis and fibromyalgia, it has also been considered as a promising non-invasive diagnostic candidate [44,45].

An analysis of a possible correlation of miR-103a-3p with other miRNAs that have shown diagnostic potential with MPM was not part of the current study but might be of interest in this context. We and others have previously tested miR-126 and miR-132-3p with MPM cases and controls [20,29]. Using data from a recent publication [46] we could not see a positive correlation between miR-103a-3p and either miR-126 or miR-132-3p.

For mesothelin and calretinin, we found significant differences in cases and controls, which remained after stratified analysis by sex and age. In the case of calretinin, significant
differences were observed within controls as expected, since most of the samples corresponded to a subsample of the study by Jiménez-Ramírez et al. [6].

Weber et al. reported that the combination of miR-103a-3p with mesothelin increased the sensitivity for MPM diagnosis up to 81% with 95% specificity, but this could not be shown in this Mexican study group. However, the previous study group was older (median age 72 years in cases and 73 years in controls) in contrast to 62 years in cases and controls in this study group, for which is also an association of miR-103a-3p with age is shown. For Mexican men >60 years an improvement of the sensitivity of miR-103a-3p could be observed. Thus, in future studies it is indicated to analyze the association of miR-103a-3p with age in more detail. Generally, further research involving a different population with a larger sample size is needed, including more female participants [25]. With respect to our results, low AUC and sensitivity for miR-103a-3p were found. In addition, miR-103a-3p in leukocytes could not differentiate between MPM cases and healthy participants, possibly due to the analyzed matrix, i.e., isolated leukocytes instead of the whole cellular fraction of blood, and the corresponding different isolation procedures. As Podolska et al. previously reported, miRNAs were sensitive to the used
isolation procedure [47]. Also, the inclusion of miR-103a-3p in any combination with mesothelin or calretinin did not substantially improve sensitivity, despite our larger study group compared to the study by Weber et al. [25]. These differences could be determined by ethnicity, along with the variability in a specific miRNA within the same population, which might hinder miRNAs’ use as a potential biomarker [48].

In the present study the combination of mesothelin and calretinin showed good sensitivities for both males and females (80.9 and 83.3, respectively). By including miR-103a-3p, the sensitivity for MPM diagnosis was not improved, because its performance as an individual marker was already negligible. In addition, the performance of mesothelin and calretinin was clearly better in the Mexican population compared to the German study group used by Weber et al., with no improvement by adding a third biomarker. It is likely that miR-103a-3p has greater utility in the Mexican population as an indicator of metabolic conditions rather than as a diagnostic biomarker of MPM. However, it would be important to evaluate this miRNA in a larger number of women in order to assess the correlation with other characteristics, such as weight, height, kidney function, glycosylated hemoglobin, etc. Finally, future studies should consider screening for miRNAs in the Mexican population to determine which miRNAs are deregulated, in order to evaluate these candidate biomarkers for MPM diagnosis.

In conclusion, the addition of miR-103a-3p to the established biomarker panel comprising of mesothelin and calretinin did not improve the diagnostic performance for MPM diagnosis. Still, miR-103a-3p levels were correlated with several characteristics not yet explored in a Mexican population, which could be useful for other purposes rather than diagnostics. Further research should aim to explore the potential clinical use of miR-103a-3p for diagnostic and
Table 3. Sensitivity and specificity of biomarkers among malignant pleural mesothelioma cases and population controls according to sex groups in a Mexican population.

| Biomarkers                  | Cut-off    | AUC (95% CI)       | Sensitivity (%) | Specificity (%) | TP (N) | TN (N) | FP (N) | FN (N) |
|-----------------------------|------------|--------------------|-----------------|-----------------|--------|--------|--------|--------|
| Mesothelin                  | 1.379 nmol/L | 0.894 (0.847–0.941) | 72.2            | 95.5            | 62     | 174    | 5      | 28     |
| Calretinin                  | 0.369 ng/ml | 0.931 (0.889–0.972) | 80.9            | 95.5            | 70     | 173    | 6      | 19     |
| miR-103a-3p                 | 1782       | 0.426 (0.355–0.497) | 4.4             | 95.5            | 4      | 171    | 8      | 86     |
| Mesothelin, calretinin, miR-103a-3p | 0.945 (0.910–0.979) | 80.9 | 95.5 | 72 | 174 | 5 | 17 |
| Mesothelin and calretinin   | 0.943 (0.909–0.977) | 80.9 | 95.5 | 71 | 174 | 5 | 18 |
| Mesothelin and miR-103a-3p  | 0.894 (0.848–0.941) | 72.2 | 95.5 | 62 | 174 | 5 | 28 |
| Calretinin and miR-103a-3p  | 0.932 (0.893–0.971) | 80.9 | 95.5 | 70 | 173 | 6 | 19 |

| Biomarkers                  | Cut-off    | AUC (95% CI)       | Sensitivity (%) | Specificity (%) | TP (N) | TN (N) | FP (N) | FN (N) |
|-----------------------------|------------|--------------------|-----------------|-----------------|--------|--------|--------|--------|
| Mesothelin                  | 1.275 nmol/L | 0.947 (0.870–1.024) | 88.9            | 97.4            | 16     | 38     | 1      | 2      |
| Calretinin                  | 0.726 ng/ml | 0.829 (0.706–0.951) | 61.1            | 97.4            | 11     | 38     | 1      | 7      |
| miR-103a-3p                 | 3082       | 0.437 (0.284–0.589) | 0               | 97.4            | 0      | 39     | 0      | 18     |
| Mesothelin, calretinin, miR-103a-3p | 0.958 (0.907–1.000) | 83.3 | 97.4 | 15 | 38 | 1 | 3 |
| Mesothelin and calretinin   | 0.951 (0.886–1.016) | 83.3 | 97.4 | 15 | 38 | 1 | 3 |
| Mesothelin and miR-103a-3p  | 0.954 (0.893–1.014) | 72.2 | 97.4 | 14 | 38 | 1 | 4 |
| Calretinin and miR-103a-3p  | 0.864 (0.758–0.970) | 55.6 | 97.4 | 11 | 36 | 3 | 7 |

TP, true positives; TN, true negatives; FP, false positives; FN, false negatives; AUC, area under the curve; CI, confidence interval. AUC, cut-offs, and sensitivity of individual biomarkers were calculated with ROC curves, at a specificity of 95%.

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Supporting information

S1 Fig. Comparison of batches. Differences between cases and controls of 103a-3p (2^−dCt) measurements performed in different years of the samples included in this study (A) and differences between miR-103a-3p (2^−dCt) measurements performed in different years of all samples included in this study (B).

(SDOCX)

S1 Table. Distribution of mesothelin and calretinin concentrations in a study of MPM cases and population controls in a Mexican population.

(DDOCX)

S2 Table. Correlations between miR-103a-3p and hematological and biochemical parameters and years of asbestos exposure in MPM cases and population controls in a Mexican population.

(DDOCX)

S3 Table. Biomarker performance in the group of Mexican men stratified by age.

(DDOCX)

S1 Dataset. Dataset of biomarker values and basic clinical parameters.

(XLSX)

prognostic purposes including chronic diseases or aberrant biochemical parameters in the Mexican population.
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References

1. IARC monographs on the evaluation of the carcinogenic risk of chemicals to man: asbestos. 1977/01/01 ed1977. 1–106 p.
2. Secretary IBA. Current asbestos ban 2019.
3. Galateau-Salle F, Churg A, Roggli V, Travis WD. The 2015 World Health Organization Classification of Tumors of the Pleura: Advances since the 2004 Classification. J Thorac Oncol. 2016; 11(2):142–54. https://doi.org/10.1016/j.jtho.2015.11.005 PMID: 26811225
4. Husain AN, Colby TV, Ordonez NG, Allen TC, Attanoos RL, Beasley MB, et al. Guidelines for Pathologic Diagnosis of Malignant Mesothelioma 2017 Update of the Consensus Statement From the International Mesothelioma Interest Group. Archives of pathology & laboratory medicine. 2018; 142(1):89–108. https://doi.org/10.5858/arpa.2017-0124-RA PMID: 28686500
5. Baas P, Scherpereel A, Nowak AK, Fujimoto N, Peters S, Tsao AS, et al. First-line nivolumab plus ipilimumab in unresectable malignant pleural mesothelioma (CheckMate 743): a multicentre, randomised, open-label, phase 3 trial. Lancet. 2021; 397(10272):375–86. https://doi.org/10.1016/S0140-6736(20)32714-8 PMID: 33485464
6. Jimenez-Ramirez C, Casjens S, Juarez-Perez CA, Raiko I, Del Razo LM, Taeger D, et al. Mesothelin, Calretinin, and Megakaryocyte Potentiating Factor as Biomarkers of Malignant Pleural Mesothelioma. Lung. 2019; 197(5):641–9. https://doi.org/10.1007/s00408-019-00244-1 PMID: 31267149
7. Santarelli L, Staffolani S, Strafella E, Nocchi L, Manzella N, Grossi P, et al. Combined circulating epigenetic markers to improve mesothelin performance in the diagnosis of malignant mesothelioma. Lung Cancer. 2015; 90(3):457–64. https://doi.org/10.1016/j.lungcan.2015.09.021 PMID: 26431916
8. Johnen G, Gawrych K, Raiko I, Casjens S, Pesch B, Weber DG, et al. Calretinin as a blood-based biomarker for mesothelioma. BMC cancer. 2017; 17(1):386. https://doi.org/10.1186/s12885-017-3375-5 PMID: 28558669
9. Aguilar-Madrid G, Pesch B, Calderon-Aranda ES, Burek K, Jimenez-Ramirez C, Juarez-Perez CA, et al. Biomarkers for Predicting Malignant Pleural Mesothelioma in a Mexican Population. International journal of medical sciences. 2016; 15(9):883–91. https://doi.org/10.7150/ijms.23939 PMID: 30008600
10. Ledda C, Senia P, Rapisarda V. Biomarkers for Early Diagnosis and Prognosis of Malignant Pleural Mesothelioma: The Quest Goes on. Cancers (Basel). 2018; 10(6).

11. Gillezeau CN, van Gerwen M, Ramos J, Liu B, Flores R, Taioli E. Biomarkers for malignant pleural mesothelioma: a meta-analysis. Carcinogenesis. 2019; 40(11):1320–31. https://doi.org/10.1093/carcin/bgz103 PMID: 31169811

12. Cristaudo A, Bonotti A, Guglielmi G, Fallahi P, Foddis R. Serum mesothelin and other biomarkers: what have we learned in the last decade? J Thorac Dis. 2018; 10(Suppl 2):S35–s9. https://doi.org/10.21037/jtd.2017.10.132 PMID: 29507805

13. Cui A, Jin XG, Zhai K, Tong ZH, Shi HZ. Diagnostic values of soluble mesothelin-related peptides for malignant pleural mesothelioma: updated meta-analysis. BMJ open. 2014; 4:e004145. https://doi.org/10.1136/bmjopen-2013-004145 PMID: 24566531

14. Pastan I, Hassan R. Discovery of mesothelin and exploiting it as a target for immunotherapy. Cancer Res. 2014; 74(12):2907–12. https://doi.org/10.1158/0008-5472.CAN-14-0337 PMID: 24824231

15. Johnen G, Burek K, Raiko I, Wichert K, Pesch B, Weber DG, et al. Prediagnostic detection of mesothelioma by circulating calretinin and mesothelin—a case-control comparison nested into a prospective cohort of asbestos-exposed workers. Sci Rep. 2018; 8(1):14321. https://doi.org/10.1038/s41598-018-32315-3 PMID: 30524313

16. Micolucci L, Akhtar MM, Olivieri F, Rippo MR, Procopio AD. Diagnostic value of microRNAs in asbestos exposure and malignant mesothelioma: systematic review and qualitative meta-analysis. Oncotarget. 2016; 7(36):58606–37. https://doi.org/10.18632/oncotarget.9686 PMID: 27259231

17. Vienberg S, Geiger J, Madsen S, Dalgaard LT. MicroRNAs in metabolism. Acta Physiol (Oxf). 2017; 219(2):346–61. https://doi.org/10.1111/apha.12681 PMID: 27009502

18. Mozzoni P, Ampollini L, Goldoni M, Alinovi R, Tiseo M, Genni L, et al. MicroRNA Expression in Malignant Pleural Mesothelioma and Asbestos: A Pilot Study. Dis Markers. 2017; 2017:9645940. https://doi.org/10.1155/2017/9645940 PMID: 28757678

19. Weber DG, Johnen G, Bryk O, Jockel KH, Bruning T. Identification of miRNA-103 in the cellular fraction of human peripheral blood as a potential biomarker for malignant mesothelioma—a pilot study. PLoS One. 2012; 7(1):e30221. https://doi.org/10.1371/journal.pone.0030221 PMID: 22253921

20. Weber DG, Gawrych K, Casjens S, Brik A, Lehner T, Taeger D, et al. Circulating miR-132-3p as a Candidate Diagnostic Biomarker for Malignant Mesothelioma. Dis Markers. 2017; 2017:9280170. https://doi.org/10.1155/2017/9280170 PMID: 28321148

21. Kirschner MB, Cheng YY, Badrian B, Kao SC, Creaney J, Edelman JJ, et al. Increased circulating miR-625-3p: a potential biomarker for patients with malignant pleural mesothelioma. J Thorac Oncol. 2012; 7(7):1184–91. https://doi.org/10.1097/JTO.0b013e3182572e83 PMID: 22617246

22. Mattoli M, Shafei AE, Azazy AE, Reda M, El-Khazragy N, Nagy AA, et al. Clinical evaluation of circulating miR-548a-3p and -20a expression in malignant pleural mesothelioma patients. Biomark Med. 2018; 12(2):129–39. https://doi.org/10.2217/bmm-2017-0224 PMID: 29338319

23. Bononi I, Comar M, Puzioz A, Stendardo M, Boschetto P, Orecchia S, et al. Circulating microRNAs found dysregulated in ex-exposed asbestos workers and pleural mesothelioma patients as potential new biomarkers. Oncotarget. 2016; 7(50):82700–11. https://doi.org/10.18632/oncotarget.12408 PMID: 27716620

24. Lo Russo G, Tessari A, Capece M, Galli G, de Braud F, Garassino MC, et al. MicroRNAs for the Diagnosis and Management of Malignant Pleural Mesothelioma: A Literature Review. Front Oncol. 2018; 8:650. https://doi.org/10.3389/fonc.2018.00650 PMID: 30622932

25. Weber DG, Casjens S, Johnen G, Bryk O, Raiko I, Pesch B, et al. Combination of MiR-103a-3p and mesothelin improves the biomarker performance of malignant mesothelioma diagnosis. PLoS One. 2014; 9(12):e114483. https://doi.org/10.1371/journal.pone.0114483 PMID: 25469901

26. Aguilar-Madrid G, Juarez-Perez CA, Markowitz S, Hernandez-Avila M, Sanchez Roman FR, Vazquez Grameix JH. Globalization and the transfer of hazardous industry: asbestos in Mexico, 1979–2000. International journal of occupational and environmental health. 2003;227–9.

27. Aguilar-Madrid G, Robles-Perez E, Juarez-Perez CA, Alvarado-Cabero I, Rico-Mendez FG, Javier KG. Case-control study of pleural mesothelioma in workers with social security in Mexico. American journal of industrial medicine. 2010; 53:241–51. https://doi.org/10.1002/ajim.20780 PMID: 20017186

28. Matias-Garcia PR, Wilson R, Mussack V, Reischl E, Waldenberger M, Gieger C, et al. Impact of long-term storage and freezethawing on eight circulating microRNAs in plasma samples. PLoS One. 2020; 15(1):e0227648. https://doi.org/10.1371/journal.pone.0227648 PMID: 31935258

29. Santarelli L, Strafella E, Staffolani S, Amati M, Emanuelli M, Santini D, et al. Association of MiR-126 with soluble mesothelin-related peptides, a marker for malignant mesothelioma. PLoS One. 2011; 6(4):e18232. https://doi.org/10.1371/journal.pone.0018232 PMID: 21483773
30. Kumar S, Vijayan M, Bhatti JS, Reddy PH. MicroRNAs as Peripheral Biomarkers in Aging and Age-Related Diseases. Prog Mol Biol Transl Sci. 2017; 146:47–94. https://doi.org/10.1016/bs.pmbts.2016.12.013 PMID: 28253991

31. Karolina DS, Tavinthan S, Arumugam A, Sepramaniam S, Pek SLT, Wong MTK, et al. Circulating miRNA Profiles in Patients with Metabolic Syndrome. The Journal of Clinical Endocrinology & Metabolism. 2012; 97(12):E2271–E6. https://doi.org/10.1210/jc.2012-1996 PMID: 23032062

32. Lu Q, Ma Z, Ding Y, Bedarida T, Chen L, Xie Z, et al. Circulating miR-103a-3p contributes to angiotensin II-induced renal inflammation and fibrosis via a SNRK/NF-kB/p65 regulatory axis. Nature Communications. 2019; 10(1):2145. https://doi.org/10.1038/s41467-019-10116-0 PMID: 31086184

33. Casjens S, Johnen G, Raiko I, Pesch B, Taeger D, Töpfner C, et al. Re-evaluation of potential predictors of calretinin and mesothelin in a population-based cohort study using assays for the routine application in clinical medicine. BMJ Open. 2021; 11(2):e039079. https://doi.org/10.1136/bmjopen-2020-039079 PMID: 33602699

34. Assmann TS, Recamonde-Mendoza M, Puñales M, Tschediel B, Canani LH, Crispim D. MicroRNA expression profile in plasma from type 1 diabetic patients: Case-control study and bioinformatic analysis. Diabetes Res Clin Pract. 2018; 141:35–46. https://doi.org/10.1016/j.diabres.2018.03.044 PMID: 29679626

35. Luo M, Xu C, Luo Y, Wang G, Wu J, Wan Q. Circulating miR-103 family as potential biomarkers for type 2 diabetes through targeting CAV-1 and SFRP4. Acta Diabetol. 2020; 57(3):309–22. https://doi.org/10.1007/s00592-019-01430-6 PMID: 31593475

36. National Institute of Statistics and Geography. Press release 645/21. 12 November 2021. Available at https://www.inegi.org.mx/contenidos/saladeperiodicos/aproposito/2021/EAP_Diabetes2021.pdf.

37. Lu Q, Ma Z, Ding Y, Bedarida T, Chen L, Xie Z, et al. Circulating miR-103a-3p contributes to angiotensin II-induced renal inflammation and fibrosis via a SNRK/NF-kB/p65 regulatory axis. Nat Commun. 2019; 10(1):2145. https://doi.org/10.1038/s41467-019-10116-0 PMID: 31086184

38. Owczarz M, Potosak J, Domaszewska-Szostek A, Kołodziej P, Kurytowicz A, Puzianowska-Kuźnicka M. Age-related epigenetic drift deregulates SIRT6 expression and affects its downstream genes in human peripheral blood mononuclear cells. Epigenetics. 2020; 15(12):1336–47. https://doi.org/10.1080/15592294.2020.1780861 PMID: 32573399

39. Cavalleri T, Angelici L, Favero C, Dionis L, Mensi C, Bareggi C, et al. Plasmatic extracellular vesicle microRNAs in malignant pleural mesothelioma and asbestos-exposed subjects suggest a 2-miRNA signature as potential biomarker of disease. PLoS One. 2017; 12(5):e0176680. https://doi.org/10.1371/journal.pone.0176680 PMID: 28472171

40. Gorčar K, Holcar M, Mavec N, Kovač V, Lenassi M, Dolžan V. Extracellular Vesicle Enriched miR-625-3p Is Associated with Survival of Malignant Mesothelioma Patients. J Pers Med. 2021; 11(10). https://doi.org/10.1093/jpm/jmaa002 PMID: 34683154

41. Zhang H, Zhu M, Shan X, Zhou X, Wang T, Zhang J, et al. A panel of seven-miRNA signature in plasma as potential biomarker for colorectal cancer diagnosis. Gene. 2019; 687:246–54. https://doi.org/10.1016/j.gene.2018.11.055 PMID: 30458288

42. Liu H, Bian QZ, Zhang W, Cui HB. Circulating microRNA-103a-3p could be a diagnostic and prognostic biomarker for breast cancer. Oncol Lett. 2022; 23(1):38. https://doi.org/10.3892/ol.2021.13156 PMID: 34966454

43. Mello-Grand M, Gregnani I, Sacchietto L, Ostano P, Zitella A, Bottoni G, et al. Circulating microRNAs combined with PSA for accurate and non-invasive prostate cancer detection. Carcinogenesis. 2019; 40(2):246–53. https://doi.org/10.1093/carcin/bgy167 PMID: 30452625

44. Papari E, Noruzinia M, Kashani L, Foster WG. Identification of candidate microRNA markers of endometriosis with the use of next-generation sequencing and quantitative real-time polymerase chain reaction. Fertil Steril. 2020; 113(6):1232–41. https://doi.org/10.1016/j.fertnstert.2020.01.026 PMID: 32482255

45. Bjerding JL, Bokarewa MI, Mannerkorpi K. Profile of circulating microRNAs in fibromyalgia and their relation to symptom severity: an exploratory study. Rheumatol Int. 2015; 35(4):635–42. https://doi.org/10.1007/s00296-014-3139-3 PMID: 25261961

46. Weber DG, Brik A, Casjens S, Burek K, Lehnert M, Pesch B, et al. Are circulating microRNAs suitable for the early detection of malignant mesothelioma? Results from a nested case-control study. BMC Res Notes. 2019; 12(1):77. https://doi.org/10.1186/s13104-019-4113-7 PMID: 30744695

47. Podolska A, Kaczkowski B, Litman T, Fredholm M, Cirera S. How the RNA isolation method can affect microRNA microarray results. Acta Biochim Pol. 2011; 58(4):535–40. PMID: 22146134

48. Vogt J, Sheinson D, Katavolos P, Imagawa H, Tseng M, Alatisis KR, et al. Variance component analysis of circulating miR-122 in serum from healthy human volunteers. PLoS One. 2019; 14(7):e0220406. https://doi.org/10.1371/journal.pone.0220406 PMID: 31348817