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PLEURAL DISEASE

Mesothelin, Calretinin, and Megakaryocyte Potentiating Factor as Biomarkers of Malignant Pleural Mesothelioma

Carmina Jiménez-Ramírez[1,2,3] · Swaantje Casjens[4] · Cuauhtémoc Arturo Juárez-Pérez[2] · Irina Raiko[4] · Luz M. Del Razo[1] · Dirk Taeger[4] · Emma S. Calderón-Aranda[1] · Hans-Peter Rihs[4] · Leonor Concepción Acosta-Saavedra[1] · Daniel Gilbert Weber[4] · Alejandro Cabello-López[2] · Beate Pesch[4] · María Dolores Ochoa-Vázquez[5] · Katarzyna Burek[4] · Luis Torre-Bouscoulet[6] · José Rogelio Pérez-Padilla[6] · Erik Marco García-Bazan[7] · Thomas Brüning[4] · Georg Johnen[4] · Guadalupe Aguilar-Madrid[8,9]

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Abstract

Purpose Malignant pleural mesothelioma (MPM) is a highly lethal cancer caused by exposure to asbestos. Currently, the diagnosis is a challenge, carried out by means of invasive methods of limited sensitivity. This is a case–control study to evaluate the individual and combined performance of minimally invasive biomarkers for the diagnosis of MPM.

Method A study of 166 incident cases of MPM and 378 population controls of Mestizo-Mexican ethnicity was conducted. Mesothelin, calretinin, and megakaryocyte potentiating factor (MPF) were quantified in plasma by ELISA. The samples were collected from 2011 to 2016.

Results Based on ROC analysis and a preset specificity of 95%, the combination of the three biomarkers reached an AUC of 0.944 and a sensitivity of 82% in men. In women, an AUC of 0.937 and a sensitivity of 87% were reached. In nonconditional logistic regression models, the adjusted ORs in men were 7.92 (95% CI 3.02–20.78) for mesothelin, 20.44 (95% CI 8.90–46.94) for calretinin, and 4.37 (95% CI 1.60–11.94) for MPF. The ORs for women were 28.89 (95% CI 7.32–113.99), 17.89 (95% CI 3.93–81.49), and 2.77 (95% CI 0.47–16.21), respectively.

Conclusions To our knowledge, this is the first study evaluating a combination of mesothelin, calretinin, and MPF, and demonstrating a sex effect for calretinin. The biomarker panel showed a good performance in a Mestizo-Mexican population, with high sensitivity and specificity for the diagnosis of MPM.

Keywords Mesothelin · Calretinin · MPF · Biomarkers · Plasma · Diagnosis

Introduction

Malignant pleural mesothelioma (MPM) is a pleural cancer caused by exposure to asbestos, which is considered a group 1 carcinogen. MPM has a long latency period and is commonly diagnosed in advanced stages [1]. In Mexico, the use of asbestos is still legal, which has caused an epidemic of MPM with 500 cases per year since 2010 [2–4]. Patients with MPM have an average survival of six months after diagnosis. The diagnosis is challenging because the signs and symptoms are nonspecific and the diagnostic methods invasive, with low sensitivity and specificity. Also, the applied immunohistochemistry tests are not standardized [5–7]. In the recent decades, research has been carried out to develop a panel of biomarkers for a noninvasive diagnosis of MPM with high sensitivity and specificity and at low costs. Proposed

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✉ Georg Johnen
johnen@ipa-dguv.de

✉ Guadalupe Aguilar-Madrid
gpeaguilarm@gmail.com;
guadalupe.aguilar@claustro.edu.mx

Extended author information available on the last page of the article

molecular markers, among others, are mesothelin, megakaryocyte potentiating factor (MPF), and calretinin [8–10]. Several studies have reported the results of different combinations of serological biomarkers to improve the diagnosis of MPM [11–14]. Recently, a prospective study with a cohort of German asbestos workers demonstrated for the first time that a combination of mesothelin and calretinin was able to detect MPM in prediagnostic plasma samples about a year before clinical diagnosis [15]. Prior to the validation in a prospective study, however, candidate markers need to be verified in the intended target population. Thus, the aim of our study was to verify mesothelin, calretinin, and MPF in a Mestizo-Mexican population.

Methods

Study population. The study was based on 166 incident cases of MPM and 378 population controls. All cases and controls were recruited in Mexico City and its metropolitan area from October 2011 to February 2016. All participants lived in and came from urban areas. Inclusion criteria were reported in detail by Aguilar-Madrid et al. [16]. Male cases were matched 1:2 and the 38 female cases, 1:3. This study was approved by the IMSS´s Scientific and Ethical Research Committee (R-2011-785-069) and by INER's Committee on Science and Bioethics in Research (C30-12). Mesothelin (limit of detection (LOD): 0.03 nmol/l, coefficient of variation (CV): 3.5%) measurements were performed using the enzyme-linked immunosorbent assay technique (ELISA) following the supplier's instructions (DY3265, R&D Systems, Minneapolis, MN). For calretinin (LOD: 0.05 ng/ml, CV: 7–10%), the commercial ELISA kit by DLD (DLD Diagnostika GmbH, Hamburg, Germany) was used, which is an improved version of the method developed by Raiko et al. [17]. MPF (based on the polypeptide $MPF_{34\text{-}288}$) was determined by ELISA (LOD: 1.30 ng/ml, CV: 6–10%) according to Raiko et al. [18].

Statistical analysis. The distributions of the biomarker concentrations were presented by medians and interquartile range (IQR). Mann–Whitney *U* and Chi-squared (χ^2) tests were used for the comparison of medians and proportions between the groups, considering a level of significance of 0.05. Receiver-operating characteristics (ROC) curves were constructed to calculate the area under the curve (AUC), and the sensitivities were calculated at a fixed specificity of 95% for each individual biomarker and in combination. Unconditional logistic regression models were constructed separately for men and women. Crude and adjusted odds ratios (OR) were calculated with a confidence interval (CI) of 95%. Spearman's correlation coefficient (r_s) and 95% CIs were used to describe rank correlations between markers. The software programs STATA 14 SE (StataCorp LLC TX, USA) and GraphPad Prism 7.0 (CA, USA) were used.

Results

Of the 166 MPM cases, 77% were men and 23% women, with 85% ($N = 141$) of the cases showing epithelioid histology. Occupational and environmental exposure to asbestos was reported for 124 (96.9%) cases and 227 (89.0%) controls in men, and for 34 (89.5%) cases and 106 (86.2%) controls in females. The median age of the MPM cases was 65 years for men and 62 years for women. The difference between the median of years of occupational exposure in male cases (14 years) and controls (19 years) was not statistically significant (Table 1).

The distribution of the median biomarker concentrations was as follows (Online Resource 1): for mesothelin 2.35 and 0.56 nmol/L in male cases and controls, respectively, and 2.06 and 0.55 nmol/L in women, respectively; for calretinin, 1.27 and 0.11 ng/ml in male cases and controls, respectively, and 1.27 and 0.26 ng/ml in women, respectively; and for MPF 50.24 and 17.18 ng/ml in male cases and controls, respectively, and 56.07 and 17.63 ng/ml in women, respectively (Table 2). Differences between cases and controls were statistically different for all markers ($p < 0.001$).

Possible influencing factors of the markers—like subtype, age, and exposure—were investigated for each sex and are summarized in Table 2 (and Online Resource 2 and 3).

Analyzing individual biomarkers using a fixed specificity of 95% revealed calretinin as the best marker for males (AUC 0.923; sensitivity 81.1%) and mesothelin for females (AUC 0.913; sensitivity 78.9%). Combination of biomarkers resulted in an improved performance. The best combination for men was calretinin/MPF (AUC 0.938; sensitivity 84.3%) and for women mesothelin/calretinin (AUC 0.947; sensitivity 86.8%). The addition of a third biomarker did not further improve the performance of the panel (Table 3 and Fig. 1).

Correlations between marker pairs are depicted in Online Resource 4; mesothelin and MPF in males showed the highest correlation ($r_s = 0.85$).

In men, adjusted ORs were for mesothelin 7.92 (95% CI 3.02–20.78), for calretinin 20.44 (95% CI 8.90–46.94), and for MPF 4.37 (95% CI 1.60–11.94). In women, adjusted ORs for mesothelin were 28.89 (95% CI 7.32–113.99), for calretinin 17.89 (95% CI 3.93–81.49), and for MPF 2.77 (95% CI 0.47–16.21) (Table 4).

The three biomarkers showed no significant difference in their medians when stratifying by stage (see Online Resource 5).

Table 1 Description of MPM cases and population controls in Mexico

Variables	Both		Men		Women	
Categorical	Cases *N* (%)	Controls *N* (%)	Cases *N* (%)	Controls *N* (%)	Cases *N* (%)	Controls *N* (%)
Total	166	378	128	255	38	123
Histologic subtype						
Epithelioid	141 (85)	–	105 (82)	–	36 (94.7)	–
Biphasic	15 (9)	–	13 (10.2)	–	2 (5.3)	–
Sarcomatoid	10 (6)	–	10 (7.8)	–	–	–
Exposure-asbestos						
No	8 (4.8)	45 (11.9)	4 (3.1)	28 (11.0)	4 (10.5)	17 (13.8)
Yes	158 (95.2)	333 (88.1)*	124 (96.9)	227 (89.0)*	34 (89.5)	106 (86.2)
Type of exposure						
Occupational						
No	50 (30.1)	187 (49.5)	19 (14.8)	86 (33.7)	31 (81.6)	101 (82.1)
Yes	116 (69.9)	191 (50.5) *	109 (85.2)	169 (66.3)*	7 (18.4)	22 (17.9)
Environmental						
No	44 (26.5)	133 (35.2)	38 (29.7)	89 (34.9)	6 (15.8)	44 (35.7)
Yes	122 (73.5)	245 (64.8)*	90 (70.3)	166 (65.1)	32 (84.2)	79 (64.3)*
Smoking						
Non-Smoker	69 (41.6)	178 (47.1)	40 (31.3)	93 (36.5)	29 (76.3)	85 (69.1)
Smoke/smoked before	97 (58.4)	200 (52.9)	88 (69.8)	162 (63.5)	9 (23.7)	38 (30.9)
Continuous	Median (IQR[a])	Median (IQR)	Median (IQR)	Median (IQR)	Median (IQR)	Median (IQR)
Age (years)	64.5 (56–71)**	59 (51–70)	65 (56–71.5)**	59 (51–71)	62 (54–70)	58 (51–66)
Years of exposure						
Occupational	12 (2.7–30.5)	17 (4–27)	14 (3–33)	19 (5–30)	3 (2–10)	5 (2–10)
Environmental	30 (16–42)	30 (18–44)	30 (17–43)	30 (18–44)	30 (15–39)	31 (17–44)

*Chi square test ($p < 0.05$)

**Mann–Whitney U test ($p < 0.05$)

[a]Interquartile range

Discussion

The diagnosis of MPM is difficult and requires histopathological confirmation based on invasively obtained tissue samples. Furthermore, limited resources in pathology will lead to late diagnoses and thus limit the benefits a patient might get from timely treatment and compensation claims [16, 15]. Biomarkers have the advantage that they are usually affordable and can be easily determined in body fluids, which are obtained minimally or noninvasively. An early diagnosis in prediagnostic blood samples, i.e., before clinical symptoms occur, would be even more beneficial because treatment could start when a tumor is in a potentially more curable stage.

Before biomarkers can be validated as markers for early detection by using a prospective cohort [15], they first should be verified in the intended target population—in the present study—a Mestizo-Mexican population. We previously examined mesothelin and calretinin in a smaller study group using an older assay format for calretinin [16, 15]. We now extended our study population, using an improved and commercially available version of the calretinin assay and added another marker, MPF.

In this study, we found that mesothelin, calretinin, and MPF exhibited a good performance for the diagnosis of MPM. Furthermore, for the first time, we verified the new assay versions for calretinin as well as MPF in a Mestizo-Mexican population that also included women.

Mesothelin has been widely studied as a diagnostic biomarker for MPM, and it is reported that its concentration in blood is higher in the epithelioid subtype than that in the sarcomatoid and biphasic subtypes [8, 19]. Reported sensitivities ranged from 19 to 69% and specificities from 88 to 100% [8, 9]. We observed that, in men, there were no significant

Table 2 Distribution of plasma concentrations of biomarkers in a study of MPM cases and population controls in Mexico

Variable	Both		Men		Women	
	Cases	Controls	Cases	Controls	Cases	Controls
	Median (IQR)	Median (IQR)	Median (IQR)	Median (IQR)	Median (IQR)	Median (IQR)
Mesothelin (nmol/L)	2.25 (1.35–4.87)	0.55 (0.41–0.77)*	2.35 (1.33–5.06)	0.56 (0.40–0.85)*	2.06 (1.35–4.49)	0.55 (0.42–0.71)*
Subtype						
Epithelioid	2.14 (1.35–4.97)		2.15 (1.31–5.63)		2.11 (1.45–4.54)**	
Biphasic	2.49 (0.60–4.02)		2.93 (2.19–4.02)		0.58 (0.44–0.73)	
Sarcomatoid	2.42 (1.36–3.48)		2.42 (1.36–3.48)		-	
Age						
≤ 60 years	2.07 (1.39–4.13)	0.49 (0.36–0.70)	2.19 (1.48–4.85)	0.48 (0.35–0.70)	1.61 (1.28–3.24)	0.52 (0.41–0.69)
> 60 years	2.41 (1.17–6.20)	0.63 (0.49–0.87) *	2.40 (1.14–6.57)	0.64 (0.48–0.92)*	2.89 (1.74–4.97)	0.60 (0.51–0.78)*
Abestos exposure						
No	2.00 (0.59–3.52)	0.59 (0.43–0.84)	1.61 (0.59–3.32)	0.57 (0.46–0.82)	2.20 (0.98–6.40)	0.60 (0.42–0.84)
Yes	2.25 (1.35–4.94)	0.55 (0.41–0.76)	2.35 (1.36–5.40)	0.56 (0.40–0.85)	2.06 (1.35–4.49)	0.53 (0.42–0.71)
Calretinin (ng/ml)	1.27 (0.48–3.00)	0.15 (0.08–0.26)*	1.27 (0.47–3.22)	0.11 (0.06–0.19)*	1.27 (0.49–2.81)	0.26 (0.17–0.39)*
Subtype						
Epithelioid	1.46 (0.54–3.31)**		1.57 (0.56–3.31)		1.38 (0.47–3.78)	
Biphasic	1.11 (0.48–3.44)		1.11 (0.48–3.44)		0.81 (0.51–1.11)	
Sarcomatoid	0.39 (0.23–0.62)		0.39 (0.23–0.62)		-	
Age						
≤ 60 years	1.25 (0.53–3.40)	0.15 (0.07–0.25)	1.53 (0.59–3.44)	0.10 (0.06–0.19)	0.87 (0.45–1.76)	0.25 (0.17–0.41)
> 60 years	1.27 (0.44–2.92)	0.15 (0.10–0.26)	1.26 (0.41–2.93)	0.13 (0.07–0.19)	1.40 (0.75–2.81)	0.28 (0.17–0.36)
Asbestos exposure						
No	3.62 (1.62–8.86)	0.18 (0.11–0.29)	3.12 (1.41–3.62)	0.14 (0.11–0.20)	8.86 (3.81–11.54)	0.32 (0.18–0.36)
Yes	1.25 (0.47–2.85)	0.14 (0.07–0.25)*	1.27 (0.47–3.00)	0.11 (0.06–0.19)*	1.14 (0.45–2.03)*	0.26 (0.17–0.39)
MPF (ng/ml)	50.81 (31.31–94.98)	17.30 (13.60–21.86)*	50.24 (31.65–94.98)	17.18 (13.76–22.28)*	56.07 (27.14–102.42)	17.63 (13.32–20.85)*
Subtype						
Epithelioid	56.18 (31.33–104.0)		55.41 (31.87–108.49)		57.11 (29.16–102.94)	
Biphasic	48.65 (24.68–69.42)		48.65 (33.92–69.42)		40.34 (24.68–56.01)	
Sarcomatoid	35.20 (22.19–48.12)		35.20 (22.19–48.12)		-	
Age						
≤ 60 years	43.96 (31.35–99.23)	15.05 (12.31–19.55)	50.24 (35.35–106.86)	15.04 (11.88–19.22)	31.25 (23.55–75.54)	15.96 (12.67–20.14)
> 60 years	54.60 (30.35–99.23)	20.00 (16.23- 24.99)*	50.12 (28.47–86.77)	20.16 (16.31–26.13)*	69.59 (39.73–104.56)*	19.73 (16.03–22.75)*
Asbestos exposure						
No	51.54 (41.59–82.02)	17.996 (15.44–21.66)	41.59 (35.90–52.94)	17.06 (14.62–20.78)	79.73 (51.54–119.36)	18.33 (16.37–24.76)
Yes	50.81 (31.17–94.98)	17.26 (13.42–21.86)	50.81 (31.35–99.23)	17.30 (13.61–22.81)	51.37 (25.09–84.08)	17.16 (13.25–20.71)

IQR Interquartile range

*Mann–Whitney *U* test ($p < 0.05$)

**Kruskal–Wallis test ($p < 0.05$)

Table 3 Sensitivities, specificities, and corresponding cut offs of individual biomarkers and their combinations in a case-control study of MPM in a Mexican population

Biomarker	Men				Women			
	Cut-off	AUC (95% CI)	Sensitivity %	Specificity %	Cut-off	AUC (95% CI)	Sensitivity %	Specificity %
Mesothelin	1.457 nmol/L	0.914 (0.881–0.947)	70.3	95.3	1.260 nmol/L	0.913 (0.840–0.985)	78.9	95.1
Calretinin (DLD)	0.330 ng/ml	0.923 (0.887–0.958)	81.1	95.3	0.671 ng/ml	0.893 (0.831–0.956)	68.4	95.1
MPF	35.726 ng/ml	0.904 (0.868–0.940)	68.5	95.3	35.293 ng/ml	0.905 (0.839–0.970)	63.2	95.1
Mesothelin + Calretinin + MPF		0.944 (0.916–0.973)	81.9	95.3		0.937 (0.876–0.998)	86.8	95.1
Mesothelin + Calretinin		0.948 (0.921–0.974)	82.7	95.3		0.947 (0.897–0.997)	86.8	95.1
Mesothelin + MPF		0.920 (0.888–0.953)	71.7	95.3		0.928 (0.862–0.994)	78.9	95.1
Calretinin + MPF		0.938 (0.906–0.970)	84.3	95.3		0.919 (0.862–0.975)	78.9	95.1

AUC, cutoffs and sensitivities of individual biomarkers were calculated based on ROC curves and a fixed specificity of 95%. The AUC, sensitivities, and specificities of the biomarkers combinations were based on nonconditional logistic regression models

AUC Area under the curve

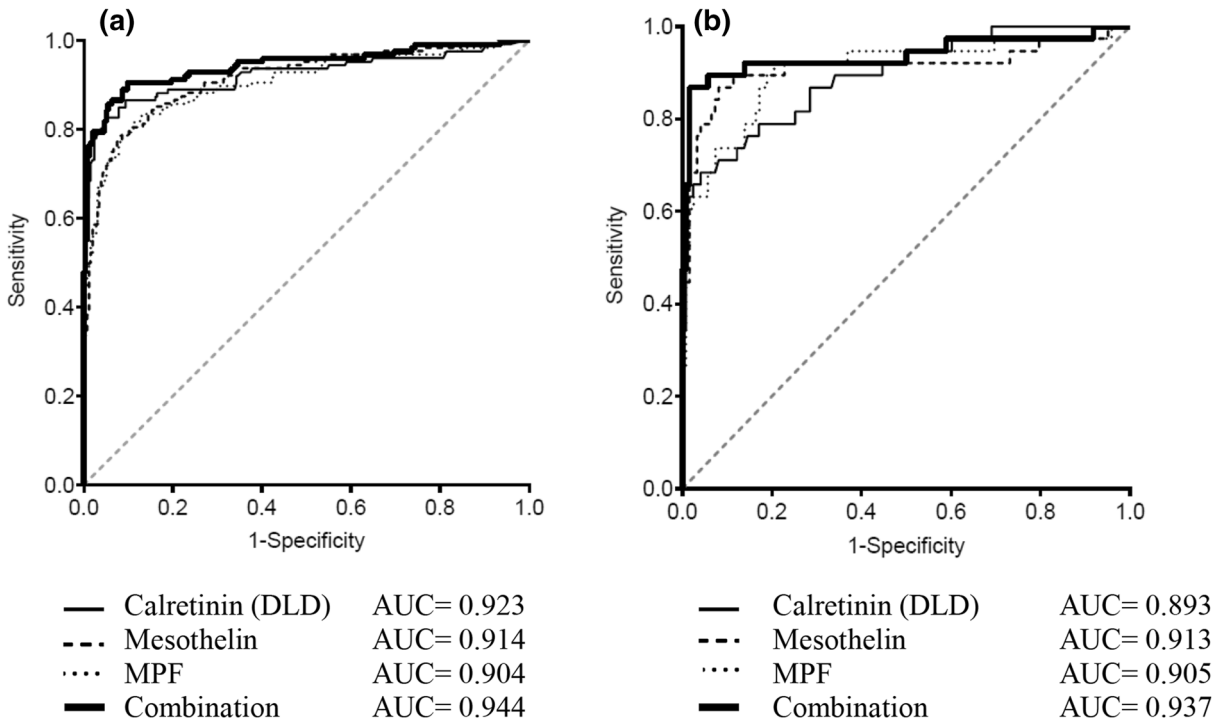

Fig. 1 Areas under the curve (AUC) for calretinin, mesothelin, and MPF in men and women. In **a** the AUC of individual and combined biomarkers in men are shown, in **b** the AUC of individual and combined biomarkers in women are shown

differences between the subtypes, which coincides with that reported in the literature for tissue staining [5].

Johnen et al. using the original method to determine plasma concentrations of calretinin by Raiko et al. reported in a German and Australian population a higher concentration of calretinin in cases that, compared to controls, was statistically significant. Regarding subtypes, however, in the Australian population, there was no significant difference in the median concentrations of calretinin in cases with the sarcomatoid subtype (0.29 ng/ml) and controls (<0.19 ng/ml), unlike the epithelioid (1.0 ng/ml) and biphasic (1.53 ng/ml) subtypes [8]. In our study, median concentrations of calretinin in sarcomatoid MPM and controls were comparable. The relatively small differences could be explained by the different study populations and the use of an improved version of the calretinin assay, DLD ELISA [15], used in the present study, which exhibited a lower detection limit. In a population-based cohort of 569 men without asbestos

Table 4 Nonconditional logistic regression models for men and women in a case-control study of MPM in a Mexican population

Models	Variable	OR[a]	95%	Adjusted OR[b]	95%
Model 1 men	Mesothelin (cutoff 1.457 nmol /L)	52.54	25.77–107.21	7.92	3.02–20.78
	Calretinin (cutoff 0.330 ng /ml)	82.28	39.80–170.08	20.44	8.90–46.94
	MPF (cutoff 35.726 ng /ml)	43 .85	21.99–87.43	4.37	1.60–11.94
Model 2 women	Mesothelin (cutoff 1.260 nmol /L)	73.12	23.58–226.81	28.89	7.32–113.99
	Calretinin (cutoff 0.671 ng /ml)	51.13	16.58–157.72	17.89	3.92–81.49
	MPF (cutoff 35.293 ng /ml)	33.14	11.57–94.95	2.77	0.47–16.21

[a]Odds ratios obtained from univariate logistic regression models with binary marker variables below or equal and above the given cutoffs as influencing factors

[b]Odds rations obtained from multiple logistic regression models with three binary markers variables as influencing factors

exposure and without malignant disease, Casjens et al. reported a median calretinin level of 0.23 ng/ml, which is somewhat higher than the median of our male controls [20]. In our study, we found significant differences in calretinin levels of controls between men and women. This difference might be explained by the presence of calretinin in other organs (e.g., uterus and ovaries) and adipose tissue, which is usually higher in women [21–23]. Whether this could have influenced the calretinin levels in the female controls has to be validated by further investigations. A limitation of our study was that the questionnaire did not include questions about the reproductive system because it was not the objective of the study, and we did not record weight and height of the participants.

For MPF, Creaney et al. reported a significant difference between MPM cases (0.23 ng/ml) and controls (0.05 ng/ml) using a commercial assay (IBL) [24]. In the sarcomatoid subtype (0.05 ng/ml), they did not find significant differences with respect to the controls. Also, Hollevoet et al. reported that MPF, using a kit by MBL [25], had significant differences in medians between cases (18.10 ng/ml) and controls (6.32 ng/ml), while the epithelioid subtype had the highest concentration of MPF (26.48 ng/ml), followed by the biphasic (17.58 ng/ml) and sarcomatoid (6.28 ng/ml) [26]. Likewise, Raiko et al. reported significant differences in median concentrations of MPF between cases (1.95 nM) and controls (0.88 nM) [18]. Our results are comparable to the published studies, with a higher concentration of MPF in epithelioid MPM, followed by biphasic and sarcomatoid, as well as a separation between cases and controls. The differences between the studies may be due to the different MPF antibodies used, the sample sizes, and different ethnicities of the populations.

We also found that age affects the concentrations of mesothelin and MPF in the control groups but no such effect for calretinin, which coincides with the report by Casjens et al. [20].

High specificity is an important characteristic of valuable biomarkers. To improve the sensitivity by marker combination, high specificity should be retained. The ROC curves of the three combined biomarkers were used to calculate sensitivities at a preset specificity of 95%. Using a combination of all three biomarkers, for men, we obtained an AUC of 0.944, with a sensitivity of 82%. In women, the performance was very similar with an AUC of 0.937 and a sensitivity of 87%. In order to improve sensitivity, others have combined mesothelin with different biomarkers such as calretinin, MPF, and/or osteopontin [8, 13, 24].

For example, Johnen et al. reported that plasma calretinin and mesothelin, by comparing male MPM cases and asbestos-exposed controls with asbestosis and/or plaques, at a specificity of 95%, reached individual sensitivities of 71% and 69%, respectively, but in combination, increased the specificity to 97% and the sensitivity to 75% [8]. The same combination had a similar performance in our study (in men), but with slightly higher sensitivities for calretinin (81%), mesothelin (70%), and the combination (83%) at 95% specificity. The markedly different individual sensitivities of calretinin (68%) and mesothelin (79%) in women in comparison to men demonstrate the importance to analyze each biomarker by sex.

For MPF, the literature reports sensitivities with a wide range, 34–90% [24, 27, 28, 25]. However, in our study, we validated for the first time, the use of an assay based on the polypeptide $MPF_{34\text{-}288}$, proposed by the Raiko et al. [18]. We obtained in men and women AUCs of 0.904 and 0.905, respectively, with a high specificity (95%) but moderate sensitivity (69% and 63%, respectively). However, when combining MPF with calretinin or mesothelin, the sensitivity increased in males and females with high specificity. Onda et al. reported that MPF increased in 51 patients (91%) but in none of their controls [27]. In addition, Creaney et al. reported a sensitivity of 34% and a specificity of 95% for MPF, comparing MPM cases with healthy controls and patients with benign asbestos-related diseases [24]. Likewise, Iwahori et al. reported a sensitivity of 74% at a specificity of 90% [25]. In another study, Creaney et al. compared two commercial methods, by MBL and IBL, to measure

MPF. They reported, at a specificity of 95%, a sensitivity of 29% for the IBL and 52% for the MBL assay, based on of 66 cases and 55 controls [10]. In contrast, Hollevoet et al. reported a sensitivity of 68% with a specificity of 97% in a group of 85 cases and 422 controls [26]. Our results are similar to those of Hollevoet et al. [26]. The differences with other reports may be due to the type of controls included, since we included healthy population controls (mostly exposed to asbestos), while other studies included controls with and without exposure that, in part, also had benign asbestos-related pathologies and used different assays and antibodies for MPF [27, 28].

With respect to the OR of our models, Cui et al. in their meta-analysis reported for mesothelin an OR of 11.84 and for MPF an OR of 36.08 [29]. In comparison, we found an adjusted OR for mesothelin of 7.9 in men and 28.9 in women, while the ORs for MPF were 4.37 and 2.77, respectively. In our previous report [16], we obtained an adjusted OR for mesothelin in men of 8.26 and in women of 21.86, which are very consistent with our current adjusted ORs for men and women, but for calretinin, we previously obtained an adjusted OR in men of 1.80 and of 1.39 in women, which were lower than those obtained in our current study, 20.4 in men and 17.9 in women. Again, these differences are probably due to the use of different calretinin assays, now showing that calretinin, based on the new assay, is the best predictor of our models. While each marker already showed a relatively good performance on its own, the two-marker combinations lead to an improved sensitivity without loss of specificity. The combination of all three markers did not lead to a further increase in sensitivity. Therefore, a combination of either calretinin and mesothelin or calretinin and MPF could be recommended. In contrast, mesothelin and MPF showed the highest correlation—and thus no significant benefit in their combination, owing to their common origin and despite their different modes of release into the bloodstream [18, 24, 27].

This is the first study to evaluate the combination of calretinin and MPF. For the first time, a sex effect has been confirmed for calretinin, which can be compensated for by a combination with MPF or mesothelin. MPF might be a cost-effective alternative to mesothelin. The next goal will be the validation of the biomarkers in a prospective high-risk cohort of asbestos-exposed individuals, which is currently being established in Mexico. Recent results from a German cohort have been encouraging [15].

In Mexico, the use of asbestos is not yet banned, and the diagnosis of MPM for the Mexican health system remains a challenge. Therefore, it is relevant to develop biomarkers for early diagnosis of MPM and to offer an alternative for a timely treatment to patients and increase their survival.

In conclusion, this is the first biomarker study for MPM performed in Mestizo-Mexican population, where the combination of mesothelin, calretinin, and/or MPF show a promising performance, resulting in specific marker combinations appropriate for men or women. We also consider it essential to develop cohort studies in populations exposed to asbestos, which will allow us to see their usefulness as probable markers for early detection of MPM in at-risk populations.

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Author Contributions CJR collected data, performed assays, analyzed data, and participated in writing the manuscript; SC analyzed data and participated in writing of the manuscript; IR performed assays and analyzed data; CAJP, LMDR, DT, ESCA, HPR, LCAS, DGW, ACL, BP, MDOV, KB, LTB, JRPP, EMGB, and TB contributed their critical opinion in the manuscript; GAM and GJ carried out the conception and design of the study, supervised the work, analyzed data, and participated in the writing of the manuscript.

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Compliance with Ethical Standards

Conflict of interest The IPA has received calretinin kits for a reduced price from DLD. Otherwise, none of the authors has any conflict of interest to declare in relation to this work.

Ethical Approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed Consent Informed consent was obtained from all individual participants included in the study.

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Affiliations

Carmina Jiménez-Ramírez[1,2,3] · Swaantje Casjens[4] · Cuauhtémoc Arturo Juárez-Pérez[2] · Irina Raiko[4] · Luz M. Del Razo[1] · Dirk Taeger[4] · Emma S. Calderón-Aranda[1] · Hans-Peter Rihs[4] · Leonor Concepción Acosta-Saavedra[1] · Daniel Gilbert Weber[4] · Alejandro Cabello-López[2] · Beate Pesch[4] · María Dolores Ochoa-Vázquez[5] · Katarzyna Burek[4] · Luis Torre-Bouscoulet[6] · José Rogelio Pérez-Padilla[6] · Erik Marco García-Bazan[7] · Thomas Brüning[4] · Georg Johnen[4] · Guadalupe Aguilar-Madrid[8,9]

1 Department of Toxicology, Center for Research and Advanced Studies of National Polytechnic Institute, CINVESTAV, Av. Instituto Politécnico Nacional 2508, Col. San Pedro Zacatenco, 07360 Mexico City, Mexico

2 Occupational Health Research Unit, Siglo XXI National Medical Center (CMNSXXI), Mexican Institute of Social Security (IMSS), Av. Cuauhtémoc 330, Col. Doctores, 06720 México, México

3 Clinical Analysis Laboratory, Traumatology Hospital "Dr. Victorio De La Fuente Narváez". High Speciality Medical Unit (UMAE), Mexican Institute of Social Security (IMSS), Av. Colector 15 s/n Esq. Av. Instituto Politécnico Nacional, Col. Magdalena de las Salinas, 07760 Mexico City, Mexico

4 Institute for Prevention and Occupational Medicine of the German Social Accident Insurance, Institute of the Ruhr-University Bochum (IPA), Bürkle-de-la-Camp-Platz 1, 44789 Bochum, Germany

5 Pneumology Service of the General Hospital, La Raza Medical Center, Mexican Institute of Social Security (IMSS), Paseo de las Jacarandas s/n, Col. La Raza, 02990 Mexico City, Mexico

6 Clinical Research, National Institute of Respiratory Diseases (INER), Calzada de Tlalpan 4502, Col. Belisario Domínguez, Sección 16, 14080 Mexico City, Mexico

7 Thorax Service, Oncology Hospital. High Speciality Medical Unit (UMAE), Siglo XXI National Medical Center (CMNSXXI), Mexican Institute of Social Security (IMSS), Av. Cuauhténmoc 330, Col. Doctores, 06720 Mexico City, Mexico

8 Research and Graduate Division, Claustro Universitario de Chihuahua, Av. División del Norte No 3104 Col. Altavista, C. P. 31200 Chihuahua, Mexico

9 Faculty of Medicine, Public Health Department, National Autonomous University of Mexico (UNAM), Av. Universidad 3000, Ciudad Universitaria, 04510 Mexico City, Mexico