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MULTICENTER STUDY OF DIFFUSE PLEURAL MESOTHELIOMA. HISTOPATHOLOGICAL AND IMMUNOHISTOCHEMICAL FEATURES

CLAUDIA POLERI[1], GABRIELA ACOSTA HAAB[2, 3], NORA FALCOFF[4], GABRIELA GUMAN[5], LILIANA DALURZO[6], ALEJANDRO IOTTI[7], MARÍA EUGENIA MARTÍN[8], GLORIA OLMEDO[9], MERCEDES RAYÁ[5], ATILIO REGINATTO[5], ANDREA WERBACH[4], GABRIELA DEMELLI[3], MARÍA JOSÉ LABANCA[6], LAURA LEGUINA[2], MARÍA FLORENCIA MORA[3]

[1]*Consultorio de Patología Torácica, Buenos Aires,* [2]*Hospital de Oncología María Curie, Buenos Aires,* [3]*San Isidro Patología, San Isidro, Provincia de Buenos Aires,* [4]*Hospital Centrángolo/Houssay, Vicente López, Provincia de Buenos Aires,* [5]*Hospital de Rehabilitación Respiratoria María Ferrer, Buenos Aires,* [6]*Hospital Italiano de Buenos Aires,* [7]*Hospital Británico, Buenos Aires,* [8]*Hospital General de Agudos Dr. E. Tornú, Buenos Aires,* [9]*Consultora en Patología Torácica, Buenos Aires, Argentina*

Abstract The pathological diagnosis of diffuse pleural mesothelioma (DPM) contributes to treatment selection and clinical trials interpretation. To know its characteristics and evaluate the viability of comprehensive pathological diagnosis of DPM in Argentina we did a retrospective descriptive study of DPM cases reported from 2009 to 2018. We analyzed 398 cases corresponding to 238 (60%) men and 160 (40%) women, median age 66 years, from surgical biopsies (78%), small biopsies (16.5%) and surgical resections (5.5%). The 77% were epithelioid (E-DPM), 12% biphasic, 10% sarcomatoid, and 4 cases transitional variant. In E-DPM the main pattern was tubular in 36% and solid in 33%. There was a second pattern in 179 cases. Considering the main pattern and the second together, 48% presented tubular subtype and 48% solid subtype. Stroma, necrosis, and nuclear score showed significant differences between E-DPM and non-epithelioid mesotheliomas. Overall tumor grade was predominantly low in E-DPM, except for 42% of the solid main pattern. We recognized the transitional variant extensively in 4 cases and focally in 8. The immunohistochemical antibody panel used included pan-cytokeratin, calretinin, WT-1, cytokeratin 5, CEA and TTF-1. The expression of cytokeratin 5, calretinin and WT-1 was lower in the sarcomatoid type (43%, 87 and 37%) than in the epithelioid type (92%, 98% and 93%). This study highlights the tumor heterogeneity of DPM that shows the diagnostic difficulty, and the feasibility of evaluating histological aggressiveness in E-DPM, B-DPM and S-DPM in our country.

Key words: pleural mesothelioma, pathology, grading mesothelioma

Resumen ***Estudio multicéntrico de características histopatológicas e inmunohistoquímicas del mesotelioma pleural difuso***. El diagnóstico patológico del mesotelioma pleural difuso (MPD) contribuye a la selección del tratamiento y a la interpretación de los ensayos clínicos. Para conocer sus características y evaluar la viabilidad del diagnóstico patológico de MPD en Argentina se realizó un estudio descriptivo retrospectivo de los casos de MPD informados de 2009 a 2018. Se analizaron 398 casos correspondientes a 238 (60%) hombres y 160 (40%) mujeres, mediana de edad de 66 años, a partir de biopsias quirúrgicas (78%), biopsias pequeñas (16.5%) y resecciones quirúrgicas (5.5%). El 77% fue epitelioide (E-MPD), 12% bifásicos, 10% sarcomatoides y 4 casos variante transicional. En E-MPD se encontró como patrón principal el tubular en 36% y el sólido en 33%. Hubo un segundo patrón en 179 casos. Considerando el principal y el segundo patrón en conjunto, el 48% presentó subtipo tubular y el 48% subtipo sólido. El estroma, la necrosis y el *score* nuclear mostraron diferencias significativas entre E-MPD y mesoteliomas no epitelioides. El grado general del tumor fue predominantemente bajo en E-MPD, a excepción del 42% del patrón principal sólido. Reconocimos la variante transicional en forma extensa en 4 casos y focalmente en 8. La expresión de citoqueratina 5, calretinina y WT-1 fue menor en el tipo sarcomatoide (43%, 87 y 37%) que en el tipo epitelioide (92%, 98% y 93%). Este estudio destaca la heterogeneidad tumoral de MPD que evidencia la dificultad en el diagnóstico y la viabilidad de evaluar la agresividad histológica en E-MPD, B-MPD y S-MPD en nuestro país.

Palabras clave: mesotelioma pleural, patología, grados de mesotelioma

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Postal address: Claudia Poleri, Tres Arroyos 296, 1414 Buenos Aires, Argentina

e-mail: claudiapoleri@gmail.com

KEY POINTS

- Diagnosis of diffuse pleural mesothelioma (DPM) type and subtypes must be precise and reproducible to stablish prognosis and treatment selection. To know its characteristics and evaluate the viability of comprehensive pathological diagnosis of DPM in Argentina we did a retrospective descriptive study of DPM cases reported from 2009 to 2018.
- This study highlights tumor heterogeneity of DPM and the feasibility of evaluating prognostic histopathological criteria included in 2021 WHO classification on pleural biopsies in E-DPM but also in B-DPM and S-DPM. Main and second pattern description have shown value in discriminating E-DPM groups with greater aggressiveness. We were able to recognize the transitional variant.

Diffuse pleural mesothelioma (DPM) is an infrequent but aggressive tumor. The Global Cancer Observatory (Globocan) estimated 30 870 new cases and 26 278 deaths in 2020 worldwide, for both sexes and all ages[1]. For Argentina it was calculated 0.23% (301 cases) of 130 878 new cancer cases, and 0.36% (252 deaths) of 70 074 cancer deaths to that year[2], without discrimination between pleural and other mesothelioma sites.

Due to the importance of the histological subtype for the selection of the treatment of patients with DPM, diagnosis must be precise and reproducible. The pathological diagnosis according to WHO 2015 classification of DPM[3] identifies epithelioid (E-DPM), sarcomatoid (S-DPM) and biphasic (B-DPM) and these categories were kept in the recent WHO Classification of Thoracic Tumors, 5th edition, as histological types with different biological aggressiveness with prognostic value[4]. There are several architectural patterns described in E-DPM and may be more than one in the same tumor, also with different prognosis[4]. The International Mesothelioma Panel carried out a reproducibility evaluation of the biphasic type in a multi-institutional study and highlighted the transitional variant of biphasic mesothelioma as an aggressive clinic pathological entity[5, 6] that, by this reason, was recently included in S-DPM subtype[4].

Other histological characteristics associated with prognosis have been proposed: necrosis, mitosis count, proliferation index (Ki67), nuclear atypia, individually or constituting scores[7-9]. Since the 14th International Mesothelioma Interest Group (IMIG) meeting[10, 11] it is recommended to incorporate histological factors (necrosis, mitosis, nuclear atypia) on the pathological reports, as a good association with prognosis has been found. In the WHO Classification of Thoracic Tumours 5th edition[4] it is recommended to report routinely nuclear score and necrosis in both biopsy and resection specimens of E-DPM to identify low or high aggressive cases.

DPM histological diagnosis needs immunohistochemical studies including mesothelial and non-mesothelial markers to assess mesothelial origin proliferation and to differentiate from other neoplasms and metastases. BAP-1 and MTAP antibodies combination is very value to indicate neoplastic or reactive mesothelial cells condition[4, 12, 13].

Despite its clinical significance, in our knowledge there are not detailed studies of the histopathological characteristics of DPM in Argentina and on immunohistochemical panels used to confirm its diagnosis. Series published are small or with only brief pathological description[14, 15].

It was our purpose to perform a DPM comprehensive morphologic and immunohistochemical review from some Pathology Laboratories in Argentina to know their histopathological characteristics and grading, to assess the feasibility of the transitional DPM diagnosis in our region, and to identify diagnostic difficulties, if there were, due to its prognostic and therapeutic implications and for the design and interpretation of clinical trial results.

Material and methods

A retrospective and multicenter study was carried out by 8 Argentinian pathology laboratories (6 from Buenos Aires City and 2 from Buenos Aires Province).

We reviewed cases with previous DPM diagnosis since 2009 to 2018, obtained from surgical biopsies or small biopsies representative of diagnostic histologic criteria, with immunohistochemistry slides available to confirm DPM diagnosis. Small biopsies were included only if they have clear mesothelial cells stromal invasion. In cases where there was both a biopsy and resection of a patient, the sample corresponding to resection was chosen.

The slides were reviewed by two or more pathologists from each laboratory without centralized review, because histopathologic parameters have been shown to be reproducible[5-7, 16].

All the potential transitional type cases and problematic or controversial cases were jointly reviewed and discussed in face-to-face meetings.

We described histological types, main and second predominant E-DPM subtypes according the 2015 WHO criteria. E-DPM subtypes analyzed were tubular, papillary, acinar, trabecular, solid, micropapilar, adenomatoid, clear cells, deciduoide, small cells, pleomorphic and rhabdoid. In S-DPM cases we indicated if desmoplastic or giant cells were present. Biphasic DPM were considered in cases with at least 10% epithelioid component in S-DPM or sarcomatoid component in E-DPM. Transitional mesothelioma was diagnosed in cases with cohesive large epithelioid cells and well-defined border without sarcomatoid characteristics. High nuclei-cytoplasmic ratio, and prominent nucleoli were present. We arbitrarily considered whether the presentation was extensive or focal ($\geq$ 5%) in combination with other subtypes.

We followed IMIG 2018 histological features recommendations: necrosis (absent, present) nuclear atypia and mitosis/10 HPF (or fields needed to reach 2 mm^2). Nuclear atypia was evaluated considering nuclear size and irregularity. It is established by the areas of the highest grade, present in > 5% of the tumor, as follows: mild (nuclei uniform in size and shape), moderate (nuclei intermediate in size between mild and severe atypia, with slight irregularity in shape), and severe (bizarre,

large nuclei of variable size with some nuclei twice as large). Scoring schemes were performed considering nuclear atypia (1 for mild, 2 for moderate, and 3 for severe atypia) and mitotic count: 1 for low (0-1 mitosis/10 HPF), 2 for intermediate (2-4 mitosis/10 HPF), and 3 for high (≥ 5 mitosis/10 HPF). Nuclear scoring scheme: total score was computed as the sum of the two parameter scores, ranging from 2 to 6. Grade I for total scores 2 or 3, grade II for total scores 4 or 5, and grade III for a total score 6. Overall tumor grade was calculated as low grade when it had nuclear score I, grade II without necrosis, high grade when it had nuclear score II with necrosis, and III with or without necrosis. We decide to evaluate nuclear score and necrosis not only in E-DPM but also in other histological types.

The stroma was categorized as desmoplastic, myxoid, with heterologous differentiation, fibrotic or without anything to specify (NOS).

Immunohistochemistry was reviewed to confirm mesothelial neoplasia and to conduct a survey of the antibody panel used in our region when the diagnosis of DPM is suspected. We considered calretinin, WT-1, AE1AE3, cytokeratin 5, CEA, TTF-1, and other antibodies used in some special cases.

To perform statistical analysis, we grouped S-DPM, transitional and biphasic-DPM in no E-DPM. We calculated frequencies and Chi-squared test or Fisher exact test and Pearson test to compare categorical variables with IBM SPSS Statistics program.

Limitations of the study: it was difficult to obtain clinical data, environmental exposure history, smoking status, staging, treatments, and evolution.

The confidentiality of the data was respected, in accordance with the Declaration of Helsinki and all its modifications and the Guide for Human Health Research (Ministry of Health of the Nation, Argentina, 2016) and Patient Rights Manual for the Health Team (Ministry of Health of the Nation, Argentina, 2021).

Results

We reviewed 398 cases corresponding to 238 (60%) men and 160 (40%) women with a median age of 66 years (24-91). Samples were obtained from surgical biopsies (78%), small biopsies (16.5%) and 5.5% from surgical resections (pleurectomy or pleural- pneumonectomy).

E-DPM was the most frequent histological type (306/398, 77%), followed by biphasic (47/398, 12%) and S-DPM (41/398, 10%). In 4/398 cases transitional DPM was found in extensive form, and in 8/398 cases focally (in 4 biphasic, 3 S-DPM and one solid E-DPM).

E-DPM main pattern was tubular in 111 (36%), solid in 101 (33%), trabecular in 37 (12%), papillary in 35 (11%), pleomorphic in 9 (3%), micropapillary in 6 (2%), adenomatoid in 6 (2%) and deciduoide in one E-DPM case.

We found a second pattern in 179 (58%) E-DPM cases. Tubular-papillary, tubular-solid and solid-tubular were the most frequent combinations (Table 1). When we considered together the main and second patterns, 48% of the E-DPM presented tubular subtype and another 48% solid subtype.

Stroma, necrosis, and nuclear score frequencies and differences between E-DPM and no E-DPM are presented in Table 2.

TABLE 1.– *Main and second pattern combinations frequencies presented in 106 epithelial-diffuse pleural mesothelioma cases*

Main/second pattern	Frequency, n (%)
Tubular/papillary	29 (16)
Tubular/solid	22 (12)
Solid/tubular	21 (12)
Solid/papillary	16 (9)
Papillary/solid	9 (5)
Trabecular/solid	9 (5)

When overall tumors were categorized, we found low grade in 233 (76%) E-DPM, in 28 (60%) B-DPM, in 26 (63%) S-DPM, and in only one transitional extensive form. We applied the same criteria to categorize more frequent E-DPM main patterns (Fig. 1).

The immunohistochemical antibody panel used was variable depending on availability at the time of diagnosis in each laboratory. In E-DPM cytokeratin 5/6, calretinin and WT-1 were employed in 84%, 91% and 79% cases, respectively. CEA was used in 66% cases, with negative results, and TTF-1 resulted negative in 250 (82%) cases performed. Pan-cytokeratin (AE1AE3) was used in 90% of S-DPM cases, all were positive. Cytokeratin 5/6, calretinin and WT-1 were employed in 85%, 98% and 76% of S-DPM, respectively. B-DPM diagnosis was done using pan-cytokeratin in 70% of these cases, cytokeratin 5/6 in 68%, calretinin in 85%, WT-1 in 79%, CEA in 64%, and TTF-1 in 74% of the cases. Expression results are described in Table 3. Extensive transitional DPM expressed cytokeratin 5 in 3/4 of the cases, calretinin and WT-1 in all 4 cases. Other antibodies, MOC-31, EMA, HBME, vimentin, thrombomodulin, and S100 protein, were also used with variable frequency, therefore their results could not be analyzed.

Discussion

This study shows DPM pathological diagnosis feasibility in Argentina, and the possibility of applying prognostic histological factors in routine practice.

Most samples were obtained by open or video-thoracoscopy biopsy which allowed good tumor representativity to analyze histology subtypes and grading, that also we could analyze in small biopsies.

It is well known that E-DPM has better prognosis than S-DPM and B-DPM, so the latter are not selected for surgery. But less is known about prognosis implications of E-DPM patterns and even less about the meaning of a second pattern. It was described tubular with a better overall survival than others, especially than pleomorphic

TABLE 2.– *Histopathological characteristics of diffuse pleural mesothelioma: comparison between epithelioid-diffuse pleural mesothelioma and non-epithelioid diffuse pleural mesothelioma shows statistically significant differences*

	E-DPM (n = 306), n (%)	no E-DPM (n = 92), n (%)	X^2
Stroma			
Desmoplasia	97 (32)	51 (55)	p < 0.001
Myxoid	38 (12)	3 (3)	
Fibrotic	123 (40)	24 (26)	
NOS	47 (16)	14 (16)	
Necrosis			
Present	61 (20)	38 (41)	p < 0.001
Absent	245 (80)	54 (59)	
Mitosis/10 HPF			
0-1	193 (63)	29 (32)	p < 0.001
2-4	81 (26.5	48 (52)	
≥ 5	32 (10.5)	15 (16)	
Nuclear atypia			
Mild	91 (30)	8 (9)	p < 0.001
Moderate	166 (54)	59 (64)	
Severe	49 (16)	25 (27)	
Nuclear score			
Grade I	184 (60)	23 (25)	p < 0.001
Grade II	98 (32)	61 (66)	
Grade III	24 (8)	8 (9)	

Fig. 1.– Overall tumor grade in more frequent diffuse epithelioid mesothelioma main patterns (n = 293)

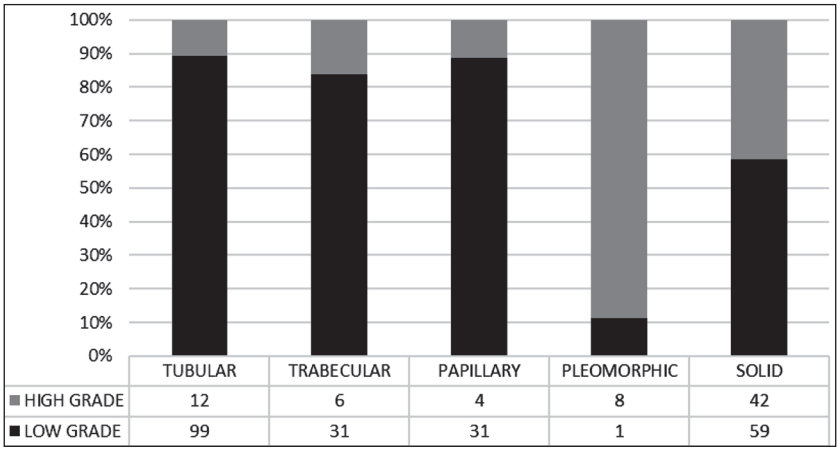

TABLE 3.– *Antibody expression frequency according to total cases made for each antibody. Positivity was evaluated in relation to number of each marker performed since the immunohistochemical panel performed was not homogeneous*

Antibody	E-DPM n = 306 n (%)	S-DPM n = 41 n (%)	B-DPM n = 47 n (%)	T-DPM n = 4 n (%)
Pancytokeratin	127/131 (97)	37/37 (100)	33/33 (100)	–
Cytokeratin 5/6	237/257 (92)	15/35 (43)	29/32 (91)	3/4
Calretinin	271/277 (98)	34/39 (87)	40/40 (100)	4/4
WT-1	224/242 (93)	11/30 (37)	35/37 (95)	4/4
CEA	0/202 (0)	0/12 (0)	0/30 (0)	0/4
TTF-1	0/250 (0)	0/16 (0)	0/35 (0)	0/4

E-DPM: epithelioid diffuse pleural mesothelioma; S-DPM: sarcomatoid diffuse pleural mesothelioma; B-DPM: biphasic diffuse pleural mesothelioma; T-DPM: transitional diffuse pleural mesothelioma (extensive form only)

E-DPM, but also than solid and micropapillary patterns[17-21]. In this way Paajanen et al[17] proposed to classify these subtypes into low-grade (trabecular, tubule-papillary) or high-grade (solid, micropapillary, pleomorphic). In the present study E-DPM was the most frequent type, with tubular and solid as the most frequent main patterns. But more than half of cases had a second pattern, showing up E-DPM tumor heterogeneity. Even though tubular/papillary combination was the most frequently found, in 48% of the E-DPM reviewed cases we found solid pattern either as main or second (combined with tubular, papillary and trabecular cases). We consider that these features can add useful data to better understand mesotheliomas biological behavior.

We found that desmoplastic stroma was present mostly in no E-DPM, but not exclusively, E-DPM had it also in a good proportion of cases (97/306). Myxoid stroma was found almost exclusively in E-DPM. Stroma characteristics should be considered in the differential diagnosis with other tumors. It also could have prognosis impact: in general patients with myxoid E-DPM seem to have a better overall survival when compared to those with epithelioid mesotheliomas[22, 23].

Kadota et al[8] studied seven nuclear features in 232 E-DPM and found that nuclear atypia and mitotic count were independent prognostic factors. These were utilized to create a three-tier nuclear grade score that correlated with clinical outcome. These results were confirmed in other studies[9] and were incorporated to recommendations in EURACAN/IASLC Proposals for Updating the Histologic Classification of Pleural Mesothelioma[24], in National Intersociety Consensus on Mesothelioma (*Academia Nacional de Medicina de Buenos Aires*)[25], and recently in WHO classification adapted to two-tier overall tumor grade including nuclear score and necrosis[4]. The objective of our study was not to analyze reproducibility, but rather a survey on the possibilities of performing the score in routine samples, therefore doubts and interpretations were discussed in face-to-face meetings even if in no case the differences would have changed the level in the score (data not shown). We were able to apply the criteria to all samples. Tubular E-DPM and solid E-DPM had necrosis and nuclear score differences which indicates their value in discriminating groups with greater aggressiveness. Bilecz et al[26] observed an association between solid and trabecular pattern with necrosis and high nuclear score. Moreover, they found in univariate analyses worst overall survival (OS) than tubular and better than pleomorphic but could not confirmed in multivariate analyses. Only pleomorphic features were able to predict OS in a study of Zhang et al[27]. Additionally, if they considered it as a function of nuclear features and included 2-level nuclear grade rather than growth pattern as a covariate, pleomorphic features remained independently prognostic. As mentioned, in this series solid pattern was frequently present, not only as the main pattern but also together with other ones. There were a few pleomorphic cases, but mostly of high grade, so we consider important to identify and report them, because at least indirectly they can suggest forms of greater aggressiveness. Although nuclear grading and necrosis were not recommended to evaluate the prognosis in non-E-DPM, we extended their application to non-E-DPM and found significant differences. Low grade overall tumor was present in most of the E-DPM while the frequency of decrease in the non-E-DPM highlighting their different biological behavior. Perhaps grading should be explored in this group.

All potential transitional cases were discussed in face-to-face meetings, and there we found that diagnostic criteria here described are useful and possible to follow

after training[4, 7, 28, 29]. Extensive form transitional cases presented necrosis, nuclear score II or III and although they are isolated cases point out their high degree of aggressiveness as described.

The immunohistochemical analysis shows up panel variability between laboratories. Although all the participants belong to the same region of Argentina, the availability of antibodies is not always the same even in a single laboratory. Pan cytokeratin (AE1 AE3) was specially used in S-DPM, but we also find it valuable to identify the sarcomatoid component in B-DPM and differentiate it from fibroblasts as previously described[29, 30] specially taking into consideration Barbieri et al[31] notice about misclassification particularly high for biphasic DPM (three-fourths of biphasic DPM at necropsy had been classified as epithelioid at VATS or surgery). Claudin-4 is considered a marker with the highest sensitivity and specificity for the diagnosis of carcinomas versus E-DPM[4,32], however it was not included in any of the panels here analyzed and to our knowledge it is not a marker used in Argentina. We believe that immunohistochemistry standardization is required in our region to improve diagnosis, preserve tissue, and optimize resources.

This study has the limitation of lacking histopathological correlation with clinical history, treatments, and survival, confirming its prognostic value, but nevertheless we believe morphological prognostic factors can be established routinely and they contribute to selecting and adapting treatments in each patient. They are critical also in clinical trials design and their results interpretation[33].

Our work remarks tumor heterogeneity of DPM and the feasibility of evaluating prognostic histopathological criteria on pleural biopsies in E-DPM, but also in B-DPM and S-DPM. Main and second pattern description have shown value in discriminating E-DPM groups with greater aggressiveness. We were able to recognize the transitional variant and given its clinical importance we must call the attention on it.

We consider that comprehensive pathological study of mesotheliomas provides useful information that allows establishing prognoses and guiding treatments with greater precision. In addition, it allows to know in detail the histological characteristics of DPM in Argentina.

Conflict of interest: None to declare

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