

Cytological diagnosis of malignant mesothelioma—improvement by additional analysis of hyaluronic acid in pleural effusions

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Abstract Cytology allows the diagnosis of malignant mesothelioma (MM) from effusions with high specificity but low sensitivity. Conversely, elevated levels of hyaluronic acid (HA) in effusions are sensitive indicators of MM, although specificity is insufficient. We studied whether the cytological diagnosis of MM could be improved by HA analysis. HA was analysed in patients with histologically confirmed MM ($n=162$), adenocarcinoma or other malignant tumours ($n=100$) and in 90 patients with benign pleural diseases. In 77 out of 162 effusions, all, and in 33 some, cytological criteria of MM were satisfied. The cut-off value of HA showing maximum diagnostic reliability (86%) regarding MM was 30 mg/l (sensitivity

87%, specificity 86%). A HA value of 100 mg/l yielded 39 and 98%, respectively. Seventy three out of 77 patients with cytological findings indicative of MM showed HA levels greater than 30 mg/l as well as 27 of 33 patients with suspicious lesions. These 100 patients were correctly recognised as having MM. The addition of HA analysis to cytology, requiring all or some criteria of MM as positive, increased sensitivity for MM from 48 to 71–91%, whereas specificity only slightly decreased to 94–96%. We conclude that the combined cytological and HA analysis of pleural effusions had the potential to improve the diagnosis of MM.

Keywords Hyaluronic acid · Malignant mesothelioma

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Introduction

Asbestos-related malignant mesothelioma (MM) shows increasing incidence over the last decade [13, 14, 19], although it is still not a common disease. Its diagnosis is usually based on clinical history with special emphasis on occupational history, thoracic imaging, pleural puncture and biopsy. In case of inconclusive cytological or histological findings, more invasive procedures such as thoracoscopy, video-assisted thoracic surgery or thoracotomy are performed. The early diagnosis of MM is difficult, and not rarely a delay of several months occurs between the onset of the symptoms and diagnosis. At the time of presentation, 54–85% of patients with MM suffer from a pleural effusion [2] as a major clinical sign that often initiates the diagnostic process.

Cytology has as a major task the distinction between reactive alterations of mesothelial cells and malignant transformations such as adenocarcinoma, MM or other

malignant tumours [17, 18]. Although the morphological criteria for MM ensure a high specificity of routine cytology (Fig. 1), sensitivity is low [1, 16, 18] as most effusions satisfy only part of the respective criteria. Hyaluronic acid (HA) has been proposed as a biochemical marker of MM as early as 1939 [12], whereby elevated levels in pleural fluid are indicative of MM. Other diseases however, such as empyema, can also lead to elevated values thus reducing the specificity of HA analysis [3, 4, 8, 15].

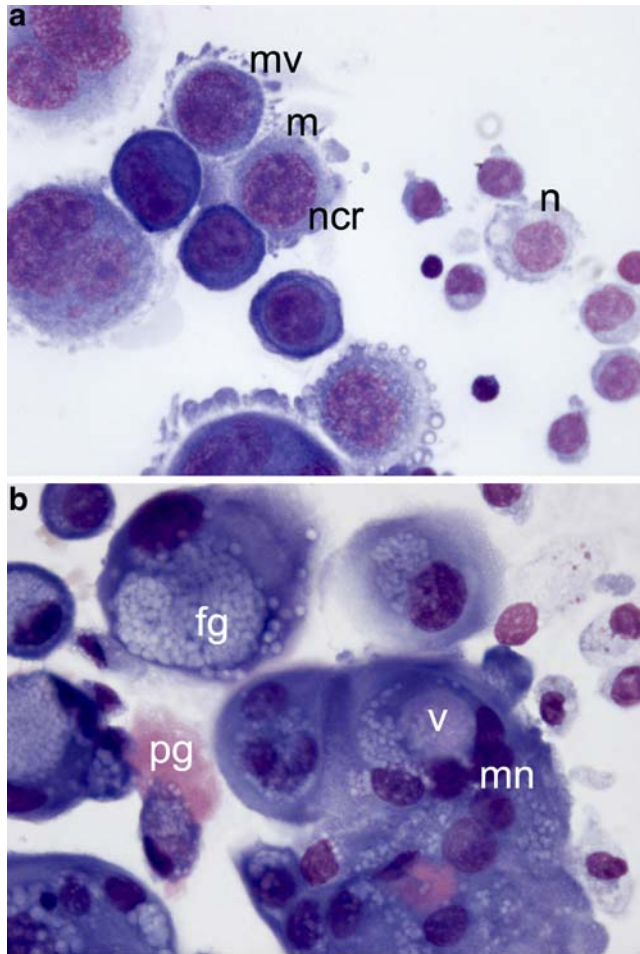


Fig. 1 Giemsa staining of cellular smears from MM (720 \times). There are several characteristic features to be observed as follows: (1) The cellular pattern is dominated by mesothelial cells looking like “caricatures” of reactive mesothelial cells; (2) bi- and multinucleate (*mn*) cells are common; (3) Giemsa smears show background staining in the form of minute pink granules (*pg*); (4) instead of two dissimilar populations as common in malignant effusions, there is a continuous transition between normal-looking mesothelial cells (*n*) and those recognised as malignant (*m*); (5) individual cells are tending to have larger, always round nuclei and a larger cytoplasm than reactive mesothelial cells with a lower nucleo-cytoplasmic ratio (*ncr*), the nucleus often being eccentrically placed nearby a single vacuole; (6) Giemsa-stained mesothelioma cells show two differently coloured areas within the cytoplasm and a pink border because of microvilli (*mv*); (7) in some cases, foamy vacuolation (*v*) and/or clusters of fat granules (*fg*) are present; (8) clusters of mesothelial cells tend to be unusually large and numerous

On the other hand, empyema can be easily identified by cytology. This suggests that a proper combination of cytology and HA analysis could take benefit from the sensitivity and specificity of the two approaches.

HA levels determined by fluid chromatography or colourimetry [4, 6, 11, 19] are known to be different from those obtained by immunological methods used today. This difference influences the choice of cut-off values. For immunological measurements, a value of 100 mg/l has been proposed [5, 7, 10, 15], although it has not been validated in a large sample. Thus, there is a need for exploring the most suitable cut-off value if the measurement of HA is evaluated either separately or in combination with routine cytology that is considered indispensable in the diagnosis of pleural effusions.

Based on these considerations, we studied whether it is possible to improve the sensitivity of the cytological diagnosis of MM while keeping its specificity by taking into account HA levels in pleural fluid. For this purpose, HA data as determined by immunoassay were analysed in a large group of patients ($n=352$, including $n=162$ patients with histologically confirmed MM). The power of cytology was additionally examined in the set of 2,516 patients from which the group taken for HA analysis had been recruited.

Materials and methods

Patients

Between 1997 and 2005, 3,289 effusions from 2,516 patients (1,694 men, 822 women; mean age, 64.6; range 15–93 years) were collected. In case of repeated assessments in one patient, the first one was taken for analysis.

HA was measured in a sample of 352 of the 2,516 patients, including 162 patients with a histologically confirmed final diagnosis of MM. For control, we randomly recruited 100 patients with histologically verified malignant tumours and 90 patients with benign pleural diseases, 77 of which were histologically proven. Based on the final diagnosis, the control patients were further classified into four groups as follows: 67 patients with adenocarcinoma (AD), 33 with other malignant tumours (OT), 20 with empyema (E) and 70 with reactive effusions (RE). Each patient’s final diagnosis was based on all available histological and/or clinical findings except cytology and HA data.

HA measurement and cytological analysis

Immediately after receipt, pleural fluid samples were centrifuged at 2,000 U/min for 5 min. Supernatants were immediately frozen and stored at -20°C . Measurements

were performed within 6 months using an immunoassay according to the manufacturer's instructions (Pharmacia, Freiburg, Germany). From the sediment, smears were prepared, fixed in 95% methanol and stained with Giemsa for microscopy. The diagnosis of "epithelial type MM" was based on well-described cytological features (Fig. 1) [14, 17, 18].

Data analysis

Median values and quartiles of HA concentrations were computed, and log-transformed HA data were compared between final diagnoses by one-way ANOVA and the Newman–Keuls post hoc test. Relative frequencies, sensitivity, specificity and diagnostic reliability were computed as usual. Sensitivity and specificity were compared between different criteria by the McNemar test or the one-sided test of proportions, if appropriate. To describe the information content of cytology from a different point of view, posterior probabilities of diagnoses according to Bayes were addi-

tionally computed [20], whereby the relative frequencies of final diagnoses were taken as estimates of prior probabilities. This type of analysis was restricted to cytology, as the result could be checked in the total population of 2,516 patients. This allowed to assess whether it was dependent on the selection of patients of the MM or control group. Using the full range of cut-off values of HA, a receiver operating characteristic (ROC) curve was computed. Statistical significance was assumed for $p < 0.05$.

Results

Cytological analysis of pleural effusions

Sensitivity and specificity

A cytological inspection showed a sensitivity of 47.5% and a specificity of 98.9% for the detection of MM, if all cytological criteria were required (Table 1). When this was

Table 1 Sensitivity and specificity of cytology, HA analysis and their combination in MM diagnosis

	Final diagnosis				Sensitivity (%)	Specificity (%)
	MM		Other			
	Yes	No	Yes	No		
Cytology alone	<i>n</i> =162		<i>n</i> =190			
All criteria for MM satisfied	77	85	2	188	47.5	98.9
Criteria only partially satisfied ^a	33/33	129/52	13/13	177/175	20.4/38.8	93.2/93.1
All or some criteria satisfied	110	52	16	175	67.9*	92.1*
HA analysis alone	<i>n</i> =162		<i>n</i> =190			
HA >30 mg/l	141	21	27	163	87.0	85.8
HA >70 mg/l	81	81	10	180	50.0	94.7
HA >100 mg/l	63	99	4	186	38.9	97.9
All cytological criteria satisfied	<i>n</i> =77		<i>n</i> =2			
HA >30 mg/l	73	4	0	2	94.8	100 ^b
HA >100 mg/l	39	38	0	2	50.6	100 ^b
All or some criteria satisfied	<i>n</i> =110		<i>n</i> =16			
HA >30 mg/l	100	10	1	15	90.9***	93.8***
HA >100 mg/l	50	60	0	16	45.5	100
HA >30 mg/l	<i>n</i> =141		<i>n</i> =27			
All criteria for MM satisfied	73	68	0	27	51.8	100.0
Criteria only partially satisfied	27	114	1	26	19.0	96.3
All or some criteria satisfied	100	41	1	26	70.9**	96.3**
HA >100 mg/l	<i>n</i> =63		<i>n</i> =4			
All or some criteria fulfilled	50	13	0	4	79.4	100.0 ^b

The table presents the results of the analyses considering either cytology and HA in separation or cytology and HA in different complementary combinations to reveal the superiority of their combined use.

* $p < 0.05$, ** $p < 0.001$ compared to "all criteria satisfied"

*** $p < 0.001$ compared to 100mg/l

^a First number referring to samples of 352 patients and the second number to the same sample without patients in whom all criteria of MM were satisfied ($n=273$).

^b Not reliable because of the small number of patients.

relaxed to the condition that either all or some criteria were satisfied, sensitivity and specificity were significantly different from the former values ($p < 0.05$ each, Table 1).

Post hoc analysis according to Bayes

This computation was first performed in patients in whom HA measurements were available ($n = 352$). The prior probability for MM increased from 46.0 to 97.4%, when all cytological criteria for MM were required, and to 72.3%, when only some criteria were required, that is, alterations were suspicious (S) of MM but inconclusive (Table 2). Conversely, the cytological diagnosis of AD or OT reduced the likelihood of MM to 16.9%, whereas the likelihood for AD and OT was raised compared to prior probabilities when cytology showed a malignancy of this type but no MM (Table 2).

The same analysis was performed in the total population of 2,516 patients with pleural effusions including those without HA measurement. This population comprised 266 patients with histologically verified MM, 557 with AD, 431 with OT, 163 with E, 1,058 with RE and 41 patients suffering from effusions of uncertain dignity (UD). Thus, the relative frequency of MM in this population was lower than in the sub-group in which HA had been measured. Correspondingly, the likelihood for MM increased from 10.6 to 93.8% when all cytological criteria for MM were satisfied (Table 3). Cytology correctly recognised 122 of the 266 patients with MM, whereas in eight patients with a cytological diagnosis of MM, the final diagnosis turned out to be OT ($n = 2$), AD or RE ($n = 3$ each). The corresponding sensitivity was 46.4% and specificity 94.1%, similar to the values observed in the sub-group of 352 patients. When only some cytological criteria were satisfied, the likelihood of MM increased to only 36.9%.

Hyaluronic acid in pleural effusions

Differences between groups

HA concentrations as measured in patients with different diagnoses are shown in Fig. 2. ANOVA and post hoc comparisons revealed HA levels to be significantly higher in MM compared to all other diagnoses ($p < 0.01$ each). In addition, HA levels in empyema were higher than in AD, OT and RE ($p < 0.05$ each). The histological sub-type could be determined in 143 patients with MM. Epithelioid MM showed the highest, and sarcomatoid MM showed the lowest values of HA; however, the differences between sub-types were not statistically significant (Table 4).

Optimal cut-off value of hyaluronic acid concentration

As illustrated by the ROC curve (Fig. 3), the highest diagnostic reliability of MM diagnosis was found at 30 mg/l, corresponding to a sensitivity of 87.0% and a specificity of 85.8% (Table 1). A cut-off value of 100 mg/l led to a lower sensitivity and a higher specificity that were significantly different between the two cut-off values ($p < 0.0001$ each).

Combination of cytology and hyaluronic acid analysis

In the sub-group of patients in whom all cytological criteria of MM were satisfied, sensitivity for MM was raised by requiring HA greater than 30 mg/l from 47.5 to 94.8% ($p < 0.0001$; Table 1); there was also a gain compared to HA analysis alone ($p = 0.03$). If all or some criteria required for MM were satisfied, sensitivity still increased from 47.5 to 90.9% ($p < 0.0001$) by using the 30 mg/l cut-off value for HA, whereas specificity was essentially unchanged

Table 2 Prior vs posterior probabilities according to Bayes (in %)

Final diagnosis	<i>n</i>	Prior	Cytological diagnosis						HA >30 mg/l
			MM	AD/OT	S	E	RE	MM/S	
MM	162	46.0	97.4	16.9	72.3	4.5	29.1	88.0	84.7
AD	67	19.0	1.3	57.7	4.3	0.0	17.2	2.4	1.8
OT	33	9.4	1.3	25.4	10.6	13.6	4.5	4.8	1.6
E	20	5.7	0.0	0.0	2.1	72.7	2.2	0.8	4.8
RE	70	19.9	0.0	0.0	10.6	9.1	47.0	4.0	7.2
Σ	352	100.0	78	71	47	22	134	125*	169

Data refer to the diagnosis of different pleural diseases by cytology in patients in whom HA had been analysed. Slashes indicate that either the first or the second cytological diagnosis was true in the respective category.

Σ Sum of absolute numbers underlying the data of the respective column (except for column "prior" where the sum of prior probabilities is given).

*Total number representing the sum of the total numbers from the MM and S column.

Table 3 Prior vs posterior probabilities according to Bayes (in %)

	Final diagnosis	n	Prior	Cytological diagnosis					
				MM	AD/OT	S	E	RE	MM/S
Data refer to the diagnosis of different pleural diseases by cytology in all patients studied over a period of 9 years.	MM	266	10.6	93.8	3.3	36.9	1.1	5.4	63.3
UD Effusion of uncertain dignity	AD	557	22.1	2.3	61.3	10.7	2.6	6.5	6.3
	OT	431	17.1	1.5	35.0	14.8	4.7	10.9	7.9
	E	163	6.5	0.0	0.0	0.8	82.1	0.4	0.4
	RE	1058	42.1	2.3	0.4	4.9	9.5	76.6	3.6
*Total number representing the sum of the total numbers from the MM and S column.	UD	41	1.6	0.0	0.0	32.0	0.0	0.1	15.5
	∑	2516	100.0	130	732	122	190	1342	252*

(Table 1). There was, however, no gain in sensitivity when introducing the 100 mg/l cut-off value. A complementary picture was obtained when performing cytology requiring all or some criteria to be satisfied in the sub-group showing HA levels greater than 30 mg/l (Table 1). Sensitivity was slightly lowered from 87.0 to 70.9% ($p < 0.0001$), but specificity raised from 85.8 to 96.3% ($p < 0.0001$). In patients demonstrating levels greater than 100 mg/l, sensitivity of MM diagnosis reached 79.4%, if all or some cytological criteria for MM were satisfied (Table 1). This was higher ($p = 0.0001$) than the sensitivity of 38.9% observed when an HA level greater than 100 mg/l was required without taking into account cytology (Table 1). To statistically compare specificities, absolute numbers were too low.

Discussion

The results of this study indicate that the combined use of cytology and HA analysis is capable of improving the

detection of MM from pleural effusions. Cytology showed a high specificity for MM, in the presence of limited sensitivity, and a cut-off value of 30 mg/l of HA turned out to be the best with regard to diagnostic reliability. When both methods were combined, HA levels allowed to correctly identify a great proportion of those samples that were cytologically suspicious but did not satisfy all of the required criteria of MM. Fully positive cytological evidence was also supported by HA analysis, corresponding to a marked increase in sensitivity, with minor change in specificity. Thus, the combined use of the two procedures appeared to be a reasonable approach in the diagnosis of MM from effusions.

In contrast to a specificity close to 100%, the sensitivity of cytology in diagnosing MM was less than 50% (Table 1). To evaluate the information contained in the data from a different point of view, we also computed the changes from before posterior probabilities as a function of cytological findings. This type of analysis was additionally performed in a large unselected group of 2,516 patients with pleural effusions that had been collected over a period of 9 years. The results obtained in the sub-group of patients having HA measurements were corroborated in this population, and a cytological finding indicative of MM raised the probability of MM from about 11 to 94%, again underlining the high specificity of the method (Table 3).

One reason for the low sensitivity of cytology seemed to be the high proportion of suspicious effusions, in which only some of the cytological criteria for MM were satisfied. This corresponds to the well-known morphological heterogeneity in MM [4, 17]. Based on this, the cytological diagnosis of MM has been depicted as not sufficiently reliable for clinical use [9], and many clinicians were reluctant to accept cytology for this purpose. In the meantime, the data base of cytologists has significantly broadened owing to the increasing incidence of MM. The high specificity of cytology, as underlined by our results, is valuable because a positive diagnosis in the presence of occupational exposure to asbestos can have important consequences beyond clinical treatment. Although immunocytochemistry has not been used in this study, such

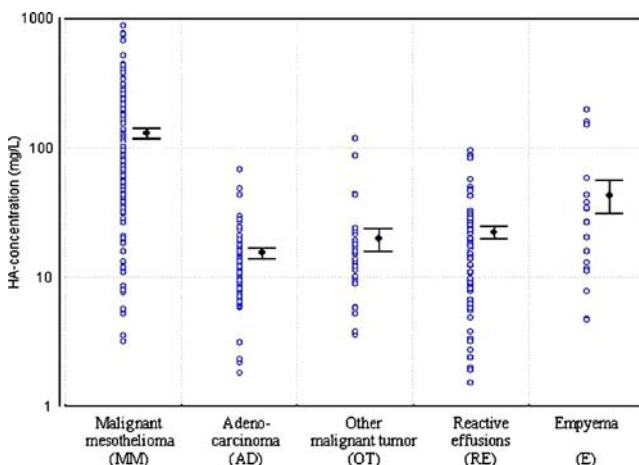


Fig. 2 Concentration of hyaluronic acid in pleural effusions of patients with different pleural diseases. Filled circles and bars indicate geometric mean values and standard errors of mean, respectively. HA levels were significantly higher in MM compared to all other diagnoses ($p < 0.01$ each). In addition, HA levels in empyema were higher than those of AD, OT and RE ($p < 0.05$, each)

Table 4 HA concentrations (mg/l) in pleural effusions for histological subtypes of MM

Subtype	<i>n</i>	Median	Lower quartile	Upper quartile	Minimum	Maximum
Epithelial	100	68.3	39.1	178.8	3.2	883.0
Sarcomatoid	6	48.8	11.7	95.2	5.2	302.0
Biphasic	37	62.0	43.6	113.3	11.6	278.0
Other	19	86.6	41.6	191.0	3.5	752.0

methods have the potential to raise sensitivity. The analysis of HA levels in pleural effusions can be considered as one step in this direction.

HA levels observed by us were similar to those found previously in patients with either MM or AD or benign pleural diseases [7]. We also confirmed that HA levels in MM were significantly higher than in AD [4, 5, 10, 15]. Little is known on HA levels in sub-types of MM as only small numbers of patients have been studied previously [19]. In our data, there was a tendency towards lower levels in sarcomatoid MM compared to other forms.

Only data on HA that have been obtained by immunological assays can be compared with our measurements. Using cut-off values of 100 mg/l of HA, sensitivity and specificity for recognising MM have been determined in small groups of 15–41 patients with MM [5, 7, 15]. Specificity ranged from 90 to 94% and sensitivity from 31 to 73%. Applying the 100-mg/l value to the data from 162 patients with MM and 190 control patients, we found estimates of sensitivity and specificity that were most similar to those reported by Fuhrman et al. [7]. Diagnostic reliability was highest at 30 mg/l, corresponding to a sensitivity of 87% and a specificity of 86%. However, the use of diagnostic reliability for identifying cut-off values has its trade off when putting special emphasis on specificity or sensitivity. Specificity at 30 mg/l was probably still too low for using HA analysis alone in the

diagnosis of MM. We took this as an entry point where cytology came into play.

When requiring all cytological criteria of MM to be positive and additionally an HA level greater than 30 mg/l, sensitivity increased markedly compared to cytology but was also raised compared to HA analysis alone (Table 1). This finding underlined the potential of HA determination if previous information from cytology was available. When testing for HA in patients with either full or partial cytological evidence for MM, sensitivity was also raised to about 91%, whereas specificity was still 94% (Table 1). If performed separately, neither cytology nor HA analysis reached this level of sensitivity at a comparable specificity of about 95%. Similar findings were obtained when adding full or partial cytological evidence for MM to the evidence obtained from the determination of HA levels. The gain in sensitivity compared to cytology was impressive in both sub-groups showing HA levels of either >30 or >100 mg/l (Table 1).

The information from the data was also reflected in the analysis of probabilities as a correlate of the positive and negative predictive value of cytology, particularly its high specificity. As judged from Tables 2 and 3, the implications for clinical practice may even have been underestimated, as the group of patients in whom HA values were available was enriched in MM compared to the set of 2,516 patients who might be considered as more of representatives for clinical practice. Among other effects, this enrichment in MM implied less room for improvement in predicting MM by the combination of the two procedures. When using the 30-mg/l cut-off value in combination with fully or partially satisfied cytological criteria, the probability of MM increased from 84 to a posterior value of 99% (data not shown). This small change reflected the already high probability of MM in patients showing elevated HA levels and conversely.

Based on these results, one might draw tentative conclusions towards a rational combination of cytology with HA analysis in clinical practice. The decision on HA measurement will rely on the clinical evidence collected and the cytological result, as far as cytology is routine in the diagnosis of pleural effusions. If cytological criteria are fully positive for MM, HA analysis could help by further increasing the already high specificity of cytology by adding assuring evidence. Nearly one third of patients with

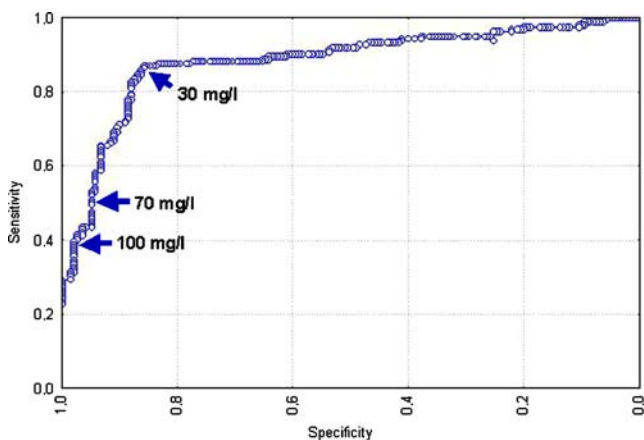


Fig. 3 ROC curve corresponding to the full range of different cut-off levels of hyaluronic acid for the diagnosis of malignant mesothelioma vs all other diseases. For orientation, three different cut-off values are indicated by arrows

MM, however, showed only partial cytological evidence in terms of suspicious lesions not satisfying all criteria of MM (Table 1). HA measurement seemed particularly advantageous in these patients. If values are greater than 30 or even 100 mg/l, patients could be considered as having MM with a high degree of certainty, and further diagnostic procedures to verify MM could be considered dispensable. This seems to be the major conclusion from our study. If cytology indicates the presence of adenocarcinoma or other tumours, HA analysis might also help owing to its high specificity for MM, if values turn out to exceed 100 mg/l (Table 1), although HA seems less informative than in the case of cytology suggesting MM. All other conditions render further (Bioptic) diagnostic procedures indispensable, as usual. The potential clinical benefits of the proposed scheme have, of course, to be assessed in independent studies. To avoid a bias arising if the results of cytology and HA analysis are used for establishing the final diagnosis, studies would still have to rely on histology in combination with clinical findings as the present analysis did.

In conclusion, the results of this study underlined the very high specificity of cytology in the diagnosis of MM. Regarding hyaluronic acid in pleural effusions, a cut-off value of 30 mg/l showed high sensitivity and a value of 100 mg/l high specificity but low sensitivity comparable to cytology. Finally, in case of positive cytological evidence for MM as well as in those cases in which only some cytological criteria of mesothelioma were satisfied, the combination of cytology and the 30-mg/l cut-off value of hyaluronic acid markedly raised sensitivity while maintaining a high specificity. Thus, the combined use of both procedures has the potential to facilitate the diagnostic process in MM without being likely to introduce unbearable expenses.

Conflict of interest statement

L Welker	None declared
M Müller	None declared
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E Vollmer	None declared
H Magnussen	None declared
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References

- Achatzy R, Beba W, Ritschler R, Worn H, Wahlers B, Macha HN, Morgan JA (1989) The diagnosis, therapy and prognosis of diffuse malignant mesothelioma. *Eur J Cardiothorac Surg* 3:445–447
- Antman K, Shemin R, Ryan L, Klegar K, Osteen R, Herman T, Lederman G, Corson J (1988) Malignant mesothelioma: prognostic variables in a registry of 180 patients, the Dana-Farber Cancer Institute and Brigham and Women's Hospital experience over two decades, 1965–1985. *J Clin Oncol* 6:147–153
- Antony VB (2003) Immunological mechanisms in pleural disease. *Eur Respir J* 21:539–544
- Atagi S, Ogawara M, Kawahara M, Sakatani M, Furuse K, Ueda E, Yamamoto S (1997) Utility of hyaluronic acid in pleural fluid for differential diagnosis of pleural effusions: likelihood ratios for malignant mesothelioma. *Jpn J Clin Oncol* 27:293–297
- Dahl IM, Solheim OP, Erikstein B, Müller E (1989) A longitudinal study of the hyaluronan level in the serum of patients with malignant mesothelioma under treatment. Hyaluronan as an indicator of progressive disease. *Cancer* 64:68–73
- Friman C, Hellström PE, Juvani M, Riska H (1977) Acid glycosaminoglycans (mucopolysaccharides) in the differential diagnosis of pleural effusion. *Clin Chim Acta* 76:357–361
- Fuhrman C, Duche JC, Chouaid C, Abd Alsamad I, Atassi K, Monnet I, Tillement JP, Housset B (2000) Use of tumor markers for differential diagnosis of mesothelioma and secondary pleural malignancies. *Clin Biochem* 33:405–410
- Hillerdal G, Lindqvist U, Engström-Laurent A (1991) Hyaluronan in pleural effusions and in serum. *Cancer* 67:2410–2414
- Martensson G (1990) Diagnosing malignant pleural mesothelioma. *Eur Respir J* 3:985–986
- Martensson G, Thylen A, Lindqvist U, Hjerpe A (1994) The sensitivity of hyaluronan analysis of pleural fluid from patients with malignant mesothelioma and a comparison of different methods. *Cancer* 73:1406–1410
- Matzel W (1985) Biochemical and cytological features of diffuse mesotheliomas of the pleura. *Arch Geschwulstforsch* 55:259–64
- Meyer K, Chaffee E (1939) Hyaluronic acid in pleural fluid associated with malignant tumour involving pleura and peritoneum. *Pro Soc Exp Biol Med* 42:797–800
- Parker C, Neville E (2003) Lung cancer * 8: management of malignant mesothelioma 1. *Thorax* 58:809–813
- Peto J, Decarli A, La Vecchia C, Levi F, Negri E (1999) The European mesothelioma epidemic. *Br J Cancer* 79:666–672
- Pettersson T, Froseth B, Riska H, Klockars M (1988) Concentration of hyaluronic acid in pleural fluid as a diagnostic aid for malignant mesothelioma. *Chest* 94:1037–1039
- Renshaw AA, Dean BR, Antman KH, Sugarbaker DJ, Cibas ES (1997) The role of cytologic evaluation of pleural fluid in the diagnosis of malignant mesothelioma. *Chest* 111:106–109
- Spriggs AI, Boddington MM (1994) Atlas of serous fluid cytopathology. A guide to the cells of pleural, pericardial, peritoneal and hydrocele fluid. In: Gresham GA (ed) Current histopathology series, vol 14. Kluwer, Dordrecht (1989)
- Stevens MW, Leong AS, Fazzalari NL, Dowling KD, Henderson DW (1992) Cytopathology of malignant mesothelioma: a stepwise logistic regression analysis. *Diagn Cytopathol* 8:333–341
- Thylen A, Hjerpe A, Martensson G (2001) Hyaluronan content in pleural fluid as a prognostic factor in patients with malignant pleural mesothelioma. *Cancer* 92:1224–1230
- Welker L, Jörres RA, Costabel U, Magnussen H (2004) Predictive value of BAL cell differentials in the diagnosis of interstitial lung diseases. *Eur Respir J* 24:1000–1006