Original Article

Aquaporin 1 Is an Independent Prognostic Factor in Pleural Malignant Mesothelioma

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BACKGROUND: Malignant mesothelioma (MM) is an aggressive cancer of serosal membranes, mostly pleura. It is related to asbestos exposure and its incidence in most industrialized countries is projected to remain stable or to increase until 2020. Prognosis remains poor. Clinical prognostic scoring systems lack precision. No prognostic tissue markers are available. Aquaporin 1 (AQP1) is a cell membrane channel involved in water transport, cell motility, and proliferation. A blocker and an agonist are available. **METHODS:** Two independent cohorts of MM were studied. Cohort 1 consisted of 80 consecutive patients who underwent radical surgery (extrapleural pneumonectomy [EPP]). Cohort 2 included 56 conservatively managed patients from another institution. Clinical information was obtained from files. Diagnoses were histologically verified. Immunohistochemical labeling for AQP1 was performed on tumor tissue and the percentage of positive cells was scored. **RESULTS:** We demonstrated expression of AQP1 in normal and neoplastic mesothelium at the apical aspect of the cell, in keeping with a role in water transport. For both cohorts, expression of AQP1 by $\geq$50% of tumor cells was associated with significantly enhanced survival (9.4 months vs 30.4 months in EPP patients and 5 months vs 15 months in conservatively treated patients). This was independent of established prognostic factors, including histologic subtype, pathologic stage, sex, and age at time of diagnosis. **CONCLUSION:** Expression of AQP1 correlated significantly with prognosis in MM, irrespective of treatment or established prognostic factors. Immunohistochemical labeling for AQP1 should be included in the routine histopathologic workup. An agonist or blocker may become useful for treatment. ***Cancer*** **2011;000:000–000.** © *2011 American Cancer Society.*

KEYWORDS: malignant mesothelioma, aquaporin 1, prognosis.

INTRODUCTION

Malignant mesothelioma (MM) is an aggressive tumor of the serosal membranes, most often affecting the pleura and related to the past inhalation of asbestos. There is a long latency interval between the first exposure to asbestos and the later discovery of the MM. Once considered a rare tumor,[1] its incidence is projected to increase in most industrialized countries until 2015-2020, and based on conservative estimates of incidence, future economic liabilities are estimated to reach around $200 billion in the United States, $80 billion in Europe, and AU$8 billion in Australia.[2-4]

MM has an extremely poor prognosis, and current treatment strategies are limited. Aggressive surgery, screening with proposed biomarkers, and various combinations of radiotherapy and chemotherapy regimens are currently being tested, but their benefit is unproven at this time.[5]

MM is often associated with large pleural effusions. Transport of water across cell membranes is not explicable by simple diffusion driven by osmotic gradients, but instead is regulated and in part facilitated by a family of transmembrane water channel proteins known as the aquaporins (AQPs).[6] At least 13 AQPs have been identified (AQP0 to AQP12)[7] that show differential expression in various tissues.[8,9]

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The lung expresses several AQPs.[10,11] AQP1 is found in vascular endothelium, AQP3 appears to be localized to the epithelium lining large air passages, and AQP4 is found in cells lining large and small airways. AQP5 has been found in alveolar epithelial cells, and its expression appears to be related to prognosis in lung cancer.[12] In experimental models and humans, AQP1 has been demonstrated in the mesothelium of the pleura and peritoneum,[13,14] and the importance of AQP1 for pleural fluid equilibrium was demonstrated in a knockout mouse model.[15] However, the exact localization of AQP1 on the mesothelial cell (apical vs lateral or basal) remains uncertain.

The study of AQPs in various disease processes is in its infancy, but AQP expression influences the pathogenesis, growth, and metastatic potential of a variety of tumors, including lung adenocarcinomas. AQP expression in stromal cells, vascular endothelium, and the neoplastic cells themselves suggests a role in carcinogenesis that has been confirmed for several types of tumors.[12,16,17]

This idea may, in the near future, have an impact on treatments, because specific AQP inhibitors/blockers and agonists have been identified recently.[17-19] Indirect modulation might also be possible via the regulatory effects of closely associated growth factors.

In preliminary experiments, we demonstrated expression of AQP1 and AQP9 in normal mesothelial cells and MM. We performed immunohistochemical screening for all AQPs for which commercial antibodies were obtainable. AQP1 was the most consistently expressed in both mesothelium and mesothelioma and therefore selected for further studies.

Genome-wide microarray studies have reported down-regulation of AQP4 in MM compared with normal pleura.[20] Microarray data indicating up-regulation of AQP1 in a subgroup of MM, based on a group of 54 samples, has been published on the National Center for Biotechnology Information online database, but these findings were not specifically discussed in the ensuing publication.[21]

We investigated the localization of AQP1 expression in mesothelial cells. We also studied the expression of AQP1 in 2 independent cohorts of patients with MM: patients treated with extrapleural pleuropneumonectomy (EPP) and patients managed conservatively. We investigated the potential role of AQP1 expression to effusion size and its relationship to survival and known prognostic factors.

MATERIALS AND METHODS

We included 2 independent MM cohorts in this study. Consecutive MM patients who underwent EPP at Royal Prince Alfred and Strathfield Private Hospitals (Sydney, Australia) from 1994 to November 2009 were reviewed and included as the testing cohort in the study. The details of the patients were kept in a prospectively collected database. The validation cohort included 56 conservatively managed patients who were diagnosed as having MM at the Department of Anatomical Pathology, Flinders Medical Centre (FMC), from 1998 to July 2010. They were considered unsuitable for aggressive surgical management due to age, advanced tumor stage, and/or poor performance status. Clinical data were obtained retrospectively and prospectively, and only those patients for whom adequate biopsy tissue and clinical data were available were included. The size of pleural effusions at the time of diagnosis was defined as follows: none, no evidence of pleural effusions; small, obliteration of costophrenic angles (<500 mL); moderate, less than half of hemothorax (500 mL to 1 L); large, more than half of hemothorax (>1 L). The histologic diagnosis of MM and histologic subtypes were determined in accordance with World Health Organization criteria.[22] Pathologic stage was determined according to the American Joint Committee on Cancer (AJCC) staging system retrospectively for the EPP cohort. No accurate clinical staging was recorded for the FMC series, and no pathologic staging was possible, because the material comprised biopsy tissue only.

Immunohistochemical Analysis

Paraffin sections were cut 4-μm thick, deparaffinized, and rehydrated before quenching with 10% H_2O_2. The sections were incubated with 1:3000 rabbit anti-human AQP1 immunoglobulin G antibody (Alpha Diagnostic, San Antonio, TX). Detection was performed using Dako Envision Plus Dual Link System Peroxidase (Dako Australia Pty. Ltd., Kingsgrove, NSW, Australia). Calretinin labeling was performed as described previously.[23] For the quantitative evaluation, the percentage of cells labeled by the antibodies was assessed visually by 2 qualified anatomic pathologists (S.K. and D.W.H.) independently, irrespective of the intensity. Only membrane labeling was considered specific, and this pattern of labeling was confirmed from 10 high-power (×400) fields. In areas of AQP1 labeling, the percentage of cells that labeled was estimated from scanning: The whole slide was scanned at ×40 magnification, because labeling was patchy in some

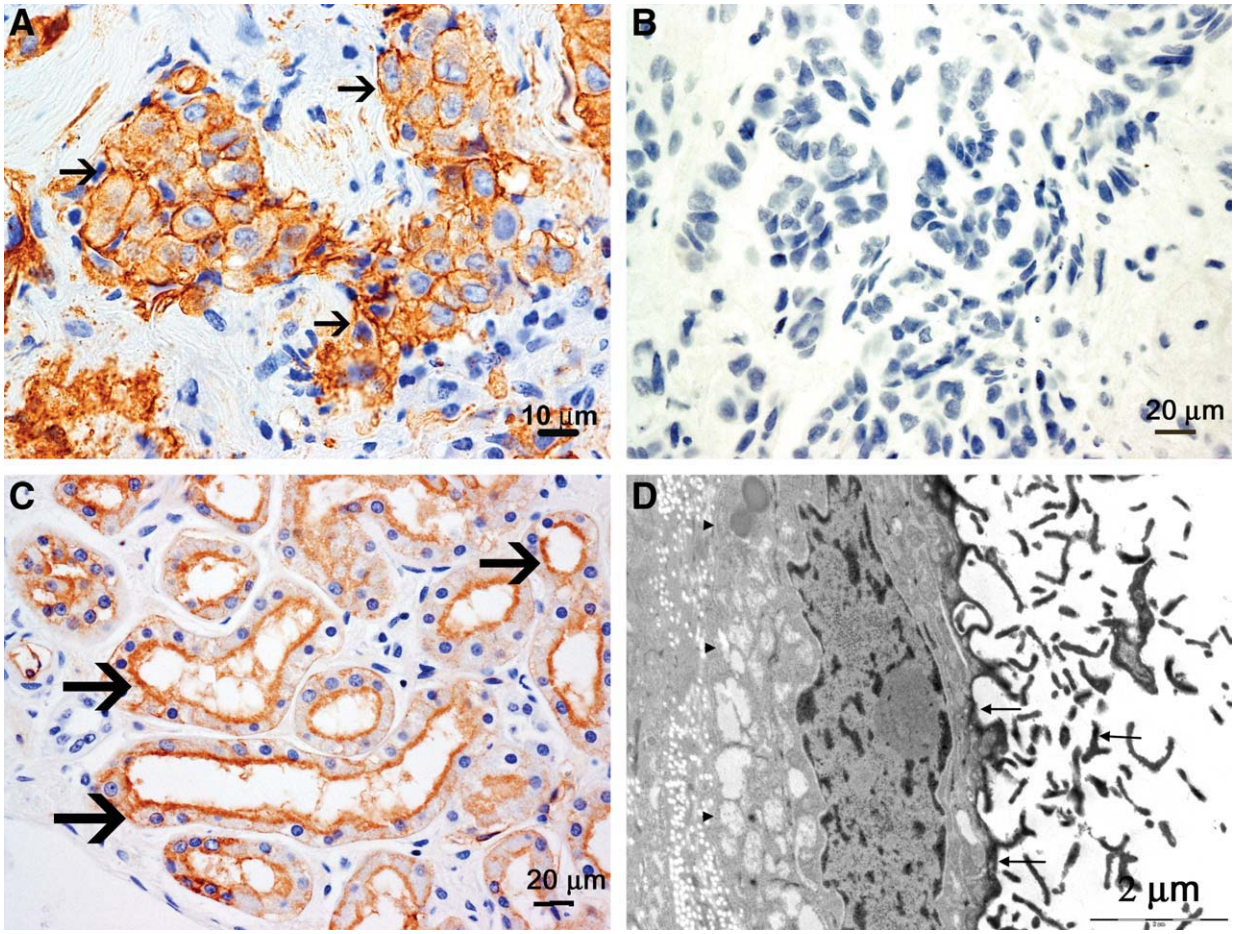

Figure 1. (A) Immunohistochemical labeling is shown for aquaporin 1 (AQP1) in an invasive malignant mesothelioma of the epithelial type, demonstrating linear membrane-related labeling (arrows). (B) A negative control demonstrating lack of labeling for AQP1 in an invasive malignant mesothelioma of the epithelial type incubated with the control primary antibody is shown. (C) A positive control demonstrating labeling for AQP1 in the renal tubular epithelium is shown (arrows). (D) In normal mesothelium, labeling is limited to the apical aspect of cells, where immunoreactivity to AQP1 is visible as dark black labeling (arrows) on microvilli.

tumors and therefore scanning of the whole slides was considered more accurate than random fields. Only labeling in tumor cells was scored; labeling in vessels (which also expressed AQP1) was not taken into account. Neither investigator was aware of the survival data when scoring was assessed. Rare discordant cases (>10% discrepancy in the percentage of labeling) were reviewed jointly, and a consensus was reached. The percentage score ranged from 0% to 100%. For the conservatively managed cohort, only 1 representative slide was assessed for labeling. These cases were diagnosed by biopsy, and for the majority, only 1 block was available. For the EPP cohort, labeling was assessed on 1 representative slide chosen as part of our previous study examining the validity of tissue microarrays in MM.[24]

Electron Microscopy

Normal human lung biopsy specimens, including the visceral pleura, were fixed overnight in 4% formaldehyde and 0.3% glutaraldehyde in 0.1 M phosphate buffer. The specimens were sectioned at 50-60 μm on a Vibratome (TPI, St. Louis, MO) and were processed for electron microscopic immunohistochemistry of AQP1 as described by Llewellyn-Smith and Minson.[25]

Ethics

The study was approved by the Human Research Ethics Committees at the Sydney South West Area Health Service–Concord Repatriation General Hospital Zone and the Human Research Ethics Committees of FMC.

Table 1. Baseline Characteristics for the 2 Cohorts of Malignant Mesothelioma Patients

Variable	EPP Series (n = 80)	FMC Series (n = 56)
Age, y, median (range)	58 (22-74)	76.5 (47-87)
Sex		
Men	63 (79)	47 (84)
Women	17 (21)	9 (16)
Histologic subtype		
Epithelial	61 (76)	23 (41)
Biphasic	19 (24)	14 (25)
Sarcomatoid	0 (0)	19 (34)
IMIG pathologic stage		
1	4 (5)	
2	16 (20)	
3	52 (65)	
4	6 (8)	
Calretinin score		
≤33%	22 (28)	
34-67%	29 (36)	
>67%	29 (36)	
AQP1 score, median (range)	65 (0-98)	15 (0-100)
<50%	32 (40)	37 (66)
≥50%	48 (60)	19 (34)
Overall survival, mo, median (range)	18.2 (11.8-24.5)	7 (5.0-9.0)

AQP1, aquaporin 1; CI, confidence interval; IMIG, International Mesothelioma Interest Group.
Data are presented as no. (%) unless stated otherwise.

Statistical Analysis

Overall survival (OS) was the primary endpoint for this study and was calculated from the date of the surgical procedure for the EPP cohort or the date of diagnosis for the FMC cohort, and the date of death or last follow-up. Patients were censored at last follow-up if still alive or lost to follow up.

Univariate Cox models were evaluated for age, sex, histologic subtype, and AQP1 score with the addition of pathologic stage and calretinin score for the EPP cohort. In a preliminary assessment of the EPP cohort for AQP1 labeling, we had found a median score of 65%. For the scoring to be robust clinically, scores of $<50\%$ versus $\geq 50\%$ were chosen. In our previous study on the EPP cohort, hemoglobin, total white cell count, and platelet count were not prognostic ($P>.05$) while calretinin score was an independent prognostic factor and hence included in the analysis in this study.[26,27] Variables with a P value $<.05$ were considered statistically significant and were examined by Kaplan-Meier curves. The univariate significant variables were then entered into a multivariate Cox model that included age, sex, and histologic subtype, as they are generally accepted prognostic factors. Association between AQP1 and histologic subtypes was assessed using the Mann-Whitney U test or the Kruskal-Wallis test where appropriate. These analyses were performed using SPSS for Windows version 17.0.

The predictive or discriminatory accuracy of AQP1, in addition to the standard age/sex/subtype variables, was investigated using the method of Schemper and Henderson,[28,29] implemented in the R package. Briefly, predictive accuracy was assessed by calculating the mean absolute difference between observed outcome and the model predictions. Explained variation was also computed and represents a measure equivalent to R^2 in linear regression. Standard errors were obtained by bootstrapping 200 samples.

RESULTS

Immunohistochemistry and Electron Microscopy

Immunohistochemistry demonstrated expression of AQP1 at the apical aspect of normal mesothelium. On the membrane of tumor cells, an apical distribution was not universally maintained and instead, circumferential labeling was commonly seen (Figure 1A-1C). The apical location of AQP1 in normal mesothelium was confirmed by immuno-electron microscopy where labeling for AQP1 was seen at the apical aspect of the cells, corresponding to the localization of microvilli on electron microscopy (Figure 1D). This location supports a role for AQP1 in fluid dynamics, as does the correlation of level of AQP1 expression with effusion size.

Patient Baseline Characteristics

From 1994 to November 2004, a total of 549 MM patients were seen in Royal Prince Alfred and Strathfield Private Hospitals: 7 had thoracoscopic biopsy alone, 245 had pleurodesis with or without biopsy, 195 had pleurectomy with or without decortications, 85 had EPP, and 17 had other procedures. Eighty patients who had EPP were included as the testing cohort, because the archival tissues were available for study. There were 56 patients in the conservatively treated FMC series. Table 1 summarizes the baseline characteristics for both the EPP cohort and FMC series. In the EPP cohort where pathologic stage was available, 6 patients were classified as having stage 4 disease on the basis of invasion to chest wall muscle or ribs, but were deemed resectable by the surgeon. The median OS was 18.2 months for the EPP cohort, with 80%

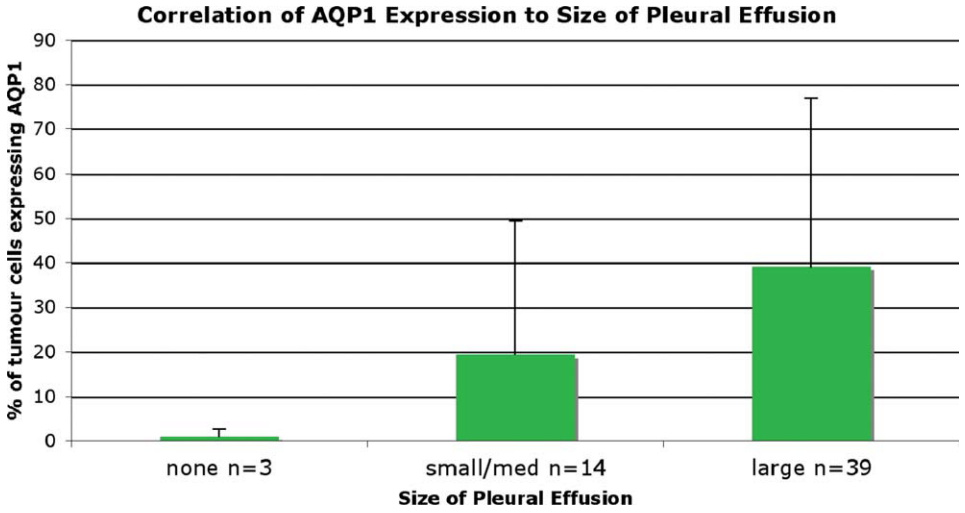

Figure 2. Correlation of aquaporin 1 (AQP1) expression to size of pleural effusions is shown. There was a trend to larger effusion size with increased AQP1 expression. The difference was not statistically significant ($P>.05$), possibly because the number of cases was too small, especially in the group with no effusions. The data shown are the mean ± standard deviation.

Table 2. Univariate and Multivariate Analysis for the Extrapleural Pneumonectomy Series

	Univariate Analysis		Multivariate Analysis	
	HR (95% CI)	*P*	HR (95% CI)	*P*
Age, y[a]	1.18 (0.91-1.53)	.21	0.79 (0.56-1.11)	.17
Sex				
Men	1.0 (reference)			
Women	0.43 (0.21-0.87)	.02	0.40 (0.18-0.93)	.03
Histologic subtype				
Epithelial	1.0 (reference)			
Biphasic	1.79 (1.02-3.14)	.04	0.87 (0.40-1.91)	.74
Pathologic stage				
1	0.18 (0.04-0.92)	.04	0.54 (0.09-3.13)	.49
2	0.23 (0.08-0.65)	.01	0.65 (0.19-2.16)	.48
3	0.33 (0.14-0.85)	.02	0.66 (0.25-1.76)	.41
4	1.0 (reference)		1.0 (reference)	
Calretinin score				
≤33%	3.77 (1.98-7.20)	<.001	3.18 (1.17-8.59)	.02
34-67%	1.64 (0.89-3.03)	.11	1.22 (0.61-2.46)	.58
≥67%	1.0 (reference)		1.0 (reference)	
AQP1 score				
<50%	2.66 (1.59-4.47)	<.001	2.14 (1.15-3.96)	.02
≥50%	1.0 (reference)		1.0 (reference)	

AQP1, aquaporin 1; CI, confidence interval; HR, hazard ratio.
[a] Increment of 10 years.

of patients deceased at the time of analysis (n = 64). The median OS was 7 months for the FMC series, with 90% of patients deceased at the time of analysis (n = 50).

The patients in the EPP cohort were younger than the patients in the FMC series (median age 58 vs 76.5 years, respectively). There was no sarcomatoid histologic subtype in the EPP cohort, whereas all 3 morphologic subtypes were represented in the FMC series. In the EPP cohort, the median OS was 23.2 vs 12.2 months for epithelial and biphasic subtype, respectively ($P = .04$). In the FMC series, median OS was also dependent on histologic subtype, with survival times of 12, 8, and 3 months for

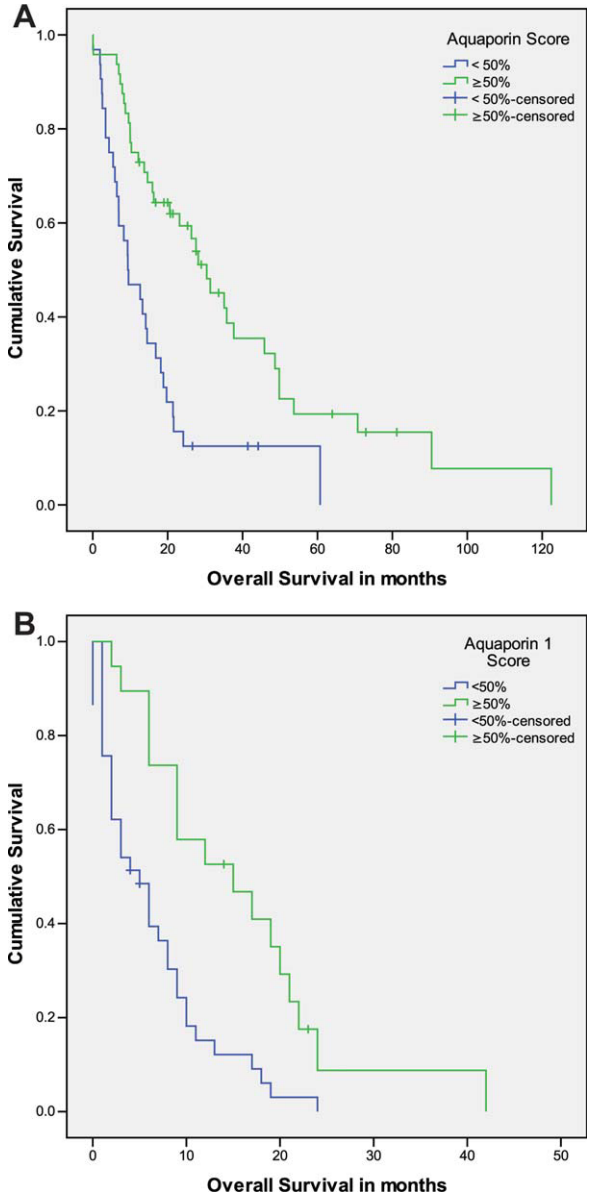

Figure 3. (A) Kaplan-Meier curve for aquaporin 1 (AQP1) for the extrapleural pneumonectomy series is shown. The median overall survival was 9.4 months (95% confidence interval [CI], 3.3-15.4) versus 30.4 months (95% CI, 21.4-39.4) for an AQP1 score <50 and ≥50%, respectively. (B) Kaplan-Meier curve for AQP1 for the Flinders Medical Centre series is shown. The median overall survival was 5 months (95% CI, 2.2-7.8) versus 15 months (95% CI, 4.3-25.7) for an AQP1 score <50 and ≥50%, respectively.

epithelial, biphasic, and sarcomatoid subtype, respectively ($P = .001$).

In the EPP cohort, 19 (24%) patients had preoperative therapy: 18 were treated with neoadjuvant chemotherapy, and 1 was treated with concurrent chemoradiation. Twenty-six (33%) patients received palliative chemotherapy at the time of recurrence. In the FMC series, no surgical procedures were performed (except for biopsy and/or pleurodesis).

AQP1 and Histologic Subtype

For the EPP cohort, the mean AQP1 score was 52.4% (standard deviation [SD] 33.4) for the epithelial subtype and 44.3% (SD 35.2) for the biphasic subtype. There was no significant difference in AQP1 expression by histologic type ($P = .43$).

For the FMC series, the mean AQP1 score was 61.8% (SD 34.6) for the epithelial subtype, 12.9% (SD 18.2) for the biphasic subtype, and 10.8% (SD 22.7) for the sarcomatoid subtype, and here the difference in expression between types was statistically significant ($P<.001$).

Potential Functional Significance of AQP1

The size of pleural effusions at the time of diagnosis was available for 46 patients in the conservatively managed FMV cohort (Figure 2). In view of the small number of samples, we grouped small- and medium-sized effusions together. There was a trend to larger effusion size with increased expression of AQP1, but the difference was not statistically significant ($P>0.05$), possibly because the number of cases was too small.

Prognostic Significance of AQP1

For the testing cohort of EPP patients, sex ($P = 0.02$), histologic subtype ($P = 0.04$), pathologic stage ($P = 0.03$), calretinin score ($P<0.001$), and AQP1 score ($P<0.001$) were all significantly associated with OS. In the multivariate Cox regression model, only sex ($P = 0.03$), calretinin score ($P = 0.02$), and AQP1 score ($P = 0.02$) remained significant (Table 2). Using the Kaplan-Meier method, the median OS was 9.4 months (95% confidence interval [CI], 3.3-15.4 months) and 30.4 months (95% CI, 21.4-39.4 months) for AQP1 scores of <50 and ≥50%, respectively (Figure 3A).

For the validation cohort of conservatively treated patients, histologic subtype ($P<.01$) and AQP1 score ($P<.01$) were significantly associated with OS. In the multivariate Cox regression model, AQP1 ($P = .02$) was the only significant variable (Table 3). Using the Kaplan-Meier method, the median OS was 5 months (95% CI, 2.2-7.8 months) and 15 months (95% CI, 4.3-25.7 months) for AQP1 scores of <50 and ≥50%, respectively (Fig. 3B).

Kaplan-Meier curves are presented in Figure 4 for the histologic subtype for both cohorts, because this is one of the most important recognized prognostic factors in

Table 3. Univariate and Multivariate Analysis in Flinders Medical Centre Series

	Univariate Analysis		Multivariate Analysis	
	HR (95% CI)	*P*	HR (95% CI)	*P*
Age, y[a]	1.18 (0.84-1.66)	.34	1.8 (0.75-1.56)	.67
Sex				
Men	1.0 (reference)		1.0 (reference)	
Women	1.34 (0.65-2.78)	.43	2.33 (0.98-4.52)	.06
Histologic subtype				
Epithelial	1.0 (reference)		1.0 (reference)	
Biphasic	2.54 (1.16-5.55)	.02	1.51 (0.62-3.69)	.37
Sarcomatoid	3.38 (1.69-6.74)	<.01	2.11 (0.98-4.52)	.06
AQP1 score				
<50%	2.71 (1.46-5.03)	<.01	2.66 (1.16-6.11)	.02
≥50%	1.0 (reference)		1.0 (reference)	

AQP1, aquaporin 1; CI, confidence interval; HR, hazard ratio.
[a] Increment of 10 years.

MM. Despite being significant in the univariate analysis for both cohorts, it was not significant in either multivariate analysis as mentioned above.

Predictive Accuracy

Inclusion of AQP1 in a multivariate Cox model together with age, sex, and histologic subtype resulted in improved predictive accuracy for both series, as well as increasing the explained variation. The effect on predictive accuracy was only moderate: in the EPP cohort, the maximum level of inaccuracy was 0.328, reducing to 0.309 when the standard prognostic variables were used, with a further reduction to 0.284 with the addition of AQP1. Similarly for the FMC series, the maximum level of inaccuracy was reduced from 0.344 to 0.298 and then 0.282 when first the standard prognostic variables and then AQP1 were added into the Cox model.

The explained variation describes how much of the variation in outcome can be explained by the variables included in the model. Using the traditional prognostic factors, only 5.8% and 13.3% of the variation in patient outcome in EPP and FMC series, respectively, can be explained. With the addition of AQP1, a much larger proportion of the variation can be explained: 13.4% and 18%, a notable improvement of 7.6% and 4.3% for EPP and FMC, respectively (Table 4).

DISCUSSION

We report for the first time the localization of AQP1 by immuno-electron microscopy to the apical surface of mesothelial cells and show that expression of AQP1 in MM is an independent prognostic factor, irrespective of the type of treatment received. Expression of AQP1 by ≥50% of tumor cells was associated with prolonged survival, with the survival difference being 21 months in the EPP group and 10 months in the conservatively treated group. This is an important observation, because prognosis in MM is particularly difficult to predict.

In addition to examining the usual multivariate Cox models, in which AQP1 was a highly significant independent variable for both cohorts, we demonstrated that use of AQP1 as a biomarker results in a moderate gain in predictive accuracy over traditional prognostic factors. This aspect of model assessment, predictive accuracy, has not been applied to biomarkers for mesothelioma previously.[30] The model with AQP1 is able to predict more accurately the prognosis of individual patients than the standard clinical model. The improvement is of moderate size, because ideally predictive inaccuracy should be 0 or very close to it. Predictive inaccuracy and explained variation are generally inversely proportional (ie, low predictive inaccuracy corresponds to a good predictive model), which explains the high proportion of variation in patient outcome in a dataset. The improved explained variation found here by including AQP1 is an indication of the prognostic value of the marker.

We believe that a difference in survival of 21 and 10 months stratified by AQP1 expression for the EPP cohort and conservatively treated group, respectively, certainly appears relevant, especially considering the effects of other known factors, and given that an improved survival in patients treated with pemetrexed—in the order of 2 months—is considered clinically significant.[30-33]

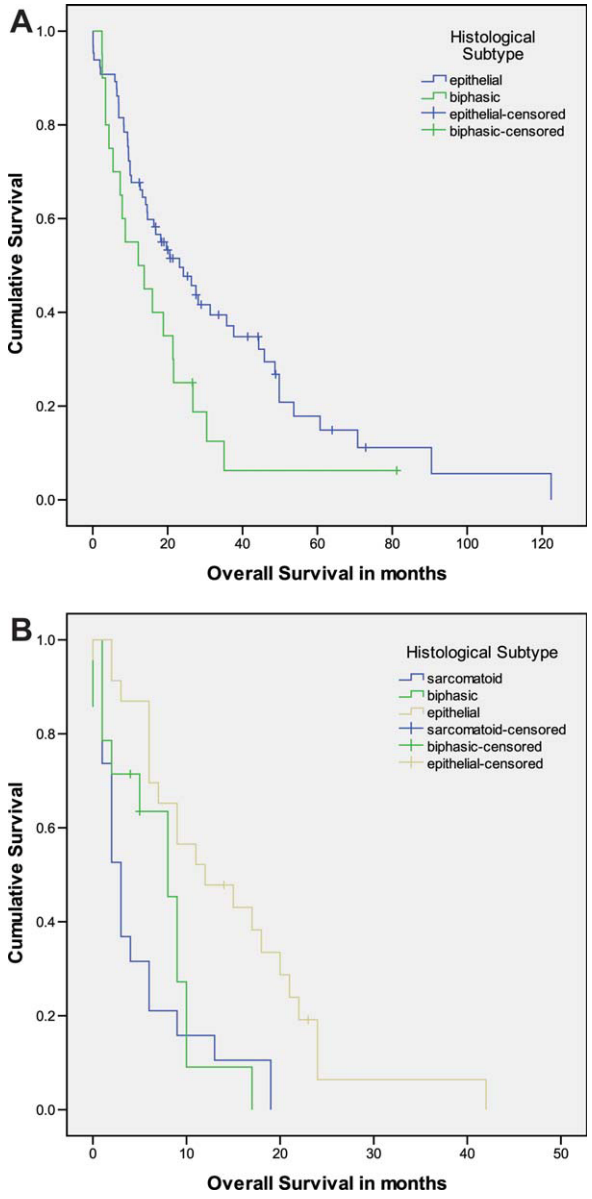

Figure 4. (A) Kaplan-Meier curve of histologic subtype for the extrapleural pneumonectomy series is shown. The median overall survival was 23.2 versus 12.2 months for the epithelial and biphasic subtype, respectively ($P = .04$). (B) Kaplan-Meier curve of histologic subtype for the Flinders Medical Centre series is shown. The median overall survival was 12, 8, and 3 months for the epithelial, biphasic, and sarcomatoid subtype, respectively ($P = .001$).

Table 4. Explained Variation and Predictive Inaccuracy for Overall Survival

Model	Predictive Inaccuracy (SE)	Explained Variation (SE)
EPP series		
No predictors	0.328	
Age+sex+subtype (model)	0.309 (0.021)	5.8% (5.0%)
Model+AQP1	0.284 (0.023)	13.4% (5.9%)
FMC series		
No predictors	0.344	
Age+sex+subtype (model)	0.298 (0.026)	13.3% (7.5%)
Model+AQP1	0.282 (0.025)	18.0% (7.6%)

AQP1, aquaporin 1; EPP, extrapleural pneumonectomy; FMC, Flinders Medical Centre; SE, standard error.

Because we have demonstrated expression of AQP1 at the apical aspect of the normal mesothelial cell, higher numbers of tumor cells expressing AQP1 may correlate with better-differentiated tumors, which could explain improved survival. However, AQP1 function appears to be altered, because there was often circumferential AQP1 expression and association of increased AQP1 expression with effusion size. Unlike most other tumors, there is no grading for MM, and tumors are only divided by histologic subtypes. The histologic subtype of MM (sarcomatoid vs biphasic vs epithelial) significantly affects survival, as confirmed in this study. Even so, in the EPP cohort the difference in survival related to AQP1 expression was independent of this only other known significant histologic indicator of prognosis in MM. There are currently no validated histologic markers included in the routine histopathology work-up of mesothelioma. Overexpression of EGFR has been associated with advanced tumor stage but not reduced survival.[34] We have previously found low expression of calretinin to be associated with poor prognosis in a subset of patients undergoing EPP.[27] Thymidylate synthase appeared to be predictive of improved overall survival in MM patients treated with pemetrexed-based chemotherapy in 2 retrospective studies, whereas excision repair cross-complementation group 1 was not associated with survival.[35-37]

The mechanism by which AQP1 affects tumor cell biology in MM, and the effects of blockade by specific blockers are the subject of our current investigations. AQP1 is also expressed by vessels, and has been shown to be up-regulated in tumor angiogenesis.[17,38] We only considered labeling of tumor cells for AQP1 as specific, and it is possible that the overall high expression of AQP1 in the tumor (ie, including desmoplastic tumor stroma with stromal vasculature, and not restricted to the tumor cells per se) may be associated with poor prognosis. Unlike other biomarkers, including AQP5, which has been found to predict outcome in lung cancer, AQP1 is not only of prognostic value, but bears great potential for clinical intervention, because both blockers and agonists are

available and have been used in animal models without adverse affects.[17]

The relatively small patient number (n = 136) in our study is a limitation, but for an uncommon tumor like MM, we believe that this is a reasonable sample size. The number of events (deaths) in the 2 cohorts was also sufficient for the number of variables examined in the multivariate models, and therefore the sample size did not compromise our statistical analysis. Furthermore, the independent prognostic value of the AQP1 was demonstrated in both cohorts of patients treated with different modality, suggesting the usefulness of this marker.

In conclusion, we found that the immunohistochemical expression of AQP1 $\geq$50% in the tumor cells was an independent predictor of longer survival in 2 independent cohorts of MM patients treated either with radical surgery or conservatively. As the relationship between patient survival and AQP1 expression appeared to be independent of the treatments received, we believe that AQP1 is an important prognostic factor to consider in MM. Assessment of the percentage of tumor cells expressing AQP1 should, in our opinion, be included into the routine diagnostic histologic work-up for MM.

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CONFLICT OF INTEREST

The authors made no disclosures.

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