Received: 3 March 2019 | Revised: 31 May 2019 | Accepted: 31 May 2019

DOI: 10.1111/cyt.12738

WILEY

ORIGINAL ARTICLE

Diagnostic value of BRCA1-associated protein-1, glucose transporter-1 and desmin expression in the discrimination between reactive mesothelial proliferation and malignant mesothelioma in tissues and effusions

Sevgen Önder[1] | Ece Özogul[1] | Deniz Koksal[2] | Sevinc Sarinc Ulasli[2] | Pinar Firat[1] | Salih Emri[2]

[1]Department of Pathology, Faculty of Medicine, Hacettepe University, Ankara, Turkey

[2]Department of Chest Diseases, Faculty of Medicine, Hacettepe University, Ankara, Turkey

Correspondence
Dr Sevgen Önder, Department of Pathology, Faculty of Medicine, Hacettepe University, 06100, Sihhiye, Ankara, Turkey.
Email: sonder@hacettepe.edu.tr

Present address
Sevinc Sarinc Ulasli, Clinics of Chest Diseases, Medisis Hospital, Ankara, Turkey

Pinar Firat, Department of Pathology, Faculty of Medicine, Koc University, Istanbul, Turkey

Salih Emri, Department of Chest Diseases, Faculty of Medicine, Yeditepe University, Istanbul, Turkey

Abstract

Objective: The aim of this study was to investigate the utility of BRCA1-associated protein-1 (BAP1), glucose transporter (GLUT)-1 and desmin expression by immunohistochemistry in the discrimination between reactive and malignant mesothelial proliferations.

Methods: A total of 88 biopsies and 30 effusions from mesothelioma cases were studied. Control groups were composed of 35 tissues and 30 cell blocks. The 88 mesothelioma cases were from 43 males and 45 females (mean age 56 years). Tumours were mostly localised to pleura (66/88, 75%) and of epithelioid histology (75/88, 85%). Cytology samples were from 17 males and 13 females (mean age 58 years), and 16 pleural and 14 peritoneal effusions. Twenty cytology cases had corresponding tissue biopsies.

Results: BAP1 loss was detected in 61/88 (69%) tissues and in 20/30 (67%) cytology samples from mesothelioma with a specificity of 100% for both sampling methods. BAP1 loss was observed more frequently in pleural and biphasic tumours. GLUT-1 immunoreactivity was identified in 54/81 (67%) and 23/25 (92%) malignant tissues and effusions, and in 6/33 (18%) and 6/30 (20%) benign tissues and effusions, respectively. Desmin loss was observed in 74/80 (92%) malignant biopsy samples, 16/21 (76%) malignant effusions and 10/34 (29%) of benign tissues, but in none of the reactive effusions. Concordance rate of results between biopsy and cytology was as follows: BAP1 20/20 (100%); GLUT-1 13/18 (72%); and desmin 10/14 (71%).

Conclusions: BAP1, GLUT-1 and desmin are useful markers in the discrimination between reactive and malignant mesothelial proliferations. BAP1 loss seems to be diagnostic for mesotheliomas both in biopsy and cytology samples.

KEYWORDS

BRCA1-associated protein-1, desmin, glucose transporter-1, immunohistochemistry, mesothelioma, reactive mesothelial proliferation

1 | INTRODUCTION

Malignant mesothelioma (MM) is an aggressive tumour that arises from mesothelial cell linings of serosal cavities, including pleural (90% of cases), peritoneal, pericardial surfaces and tunica vaginalis. Asbestos is the main aetiological factor, and is responsible for about 80% of MM. The incidence of MM is high in Turkey, which is mainly a result of exposure to widely-found environmental asbestos, as well as erionite, a fibrous zeolite which is endemic in a certain rural regions of Cappadocia.[1-3] A genetic background and familial cases have also been reported in a subset of MM patients.[4,5]

The diagnosis of mesothelioma, particularly discrimination from reactive mesothelial proliferations (RMP), has been a diagnostic dilemma in pathology practice. Although cytomorphological criteria favouring malignancy are well-established, their value may be limited in small biopsies and effusions. Thus, ancillary studies such as immunohistochemistry (IHC) and molecular tests are recommended in all available cases, as highlighted and updated in pathology and cytology guidelines.[6,7] Although the usefulness of IHC has been well-proven, diagnostic accuracy rates of IHC markers vary and are not yet perfect. Diagnosis of MM can be improved further by the combined use of IHC antibodies or use of other ancillary tests such as the detection of homozygous deletion of p16INK gene by fluorescent in situ hybridisation (FISH) technique.[8] However, IHC still remains the method of choice in daily practice as it is easily available, less labour intensive and relatively inexpensive.

Several IHC markers, such as glucose transporter (GLUT)-1 and desmin, have been among the preferred antibodies due to their relatively higher diagnostic performances. Recently, demonstration of nuclear protein expression loss of BRCA1-associated protein 1 (BAP1) gene in mesothelial cells has emerged as a marker of malignancy with a very high specificity.

BAP1 is a nuclear-localised deubiquitinating enzyme that regulates gene expression, transcription, and DNA repair and acts as a tumour suppressor by enhancing BRCA1-mediated inhibition of cellular proliferations.[9] Loss or inactivation of BAP1, which may occur as a result of chromosomal deletions involving the BAP1 gene locus (3p21.1) or due to sequence variation in the BAP1 gene, has been shown to be associated with several tumours, including MM.[10] Germline mutations were also identified in some family clusters and in very few sporadic MM cases.[11] Recently, IHC evaluation of BAP1 expression has emerged as a diagnostic tool in mesotheliomas.

GLUT-1 is one of 14 members of the mammalian facilitative GLUT family of passive glucose carriers that is not detectable in a large proportion of cells from normal tissues and benign lesions. In contrast, by maintaining energy supplies, GLUT-1 has been hypothesised to allow survival advantage to malignant cells, and its expression has been suggested to be a marker of various malignancies, including MM. IHC for GLUT-1, alone or in combination, has been among the mostly used markers in the differential diagnosis between RMP and MM.[12-14]

Desmin is an intracellular intermediate filament that is also expressed in mesothelial cells but more commonly in benign rather than malignant mesothelium, and loss of desmin expression has been used as a marker favouring mesothelioma.[15-17]

In this study, we evaluated the diagnostic performance of BAP1, GLUT-1 and desmin expression in the discrimination between RMP and MM in surgical biopsy materials and effusion samples.

2 | METHODS

2.1 | Case selection and process of the samples

Among mesothelioma cases that have been diagnosed at the Department of Pathology at Hacettepe University, Faculty of Medicine between 2001 and 2018, a total of 88 tissue biopsies and 30 cytology samples were included in the study. Cases were selected on the basis of availability of adequate sample for IHC analysis.

Among 88 MM patients with tissue biopsies, 43 were male and 45 were female. Patients were aged 19-88 years (mean 56; median 57). Based on the clinical history, 84 patients were habitants of known environmental asbestos regions and four patients were from erionite villages in Cappadocia. The location of the tumours were pleura (66/88), peritoneum (20/88), pericardium (1/88) and tunica albuginea (1/88). Histologically, 75 were epithelioid and 12 were biphasic types; one tumour was diagnosed as well-differentiated papillary mesothelioma. A series of tissue microarrays (TMAs) with 4-mm-diameter tissue cores were constructed from 69 cases; 19 cases that were not available for TMA were evaluated on whole mount sections.

Thirty cytology samples from 16 pleural and 14 peritoneal effusions in the study were composed of 26 cell blocks (CBs) and four cytospin preparations. Twenty cases, including three cytospins without a CB, had available corresponding tissue biopsies and were included in the cohort. MM diagnosis was confirmed by IHC with calretinin, WT-1, D2-40, GLUT-1 and desmin. One cytospin case was diagnosed and treated as MM based on clinical data, DNA ploidy studies and cytomorphology. Patients were 17 males and 13 females with ages ranging between 20 and 80 years (mean 58; median 59). Control groups were composed of 35 biopsies and 30 CBs from pleural or peritoneal effusions obtained from patients who underwent sampling for non-neoplastic conditions.

Paraffin sections were cut at 0.4 μm thick, then deparaffinised and put in an automated IHC stainer (Leica BOND-MAX™) and stained with BAP1 (Santa Cruz Biotechnology; clone C-4; dilution 1:150), GLUT-1 (Cell Marque; rabbit polyclonal; dilution 1:100) and desmin (Novocastra; clone DE-R-11; dilution 1:100). For preparing cell blocks, about 10 mL of each sample was centrifuged at 374 *g* for 5 minutes. The cell pellet was fixed in 10% neutral buffered formalin. The tissue block was processed and applied IHC as described.

	BAP1 loss (%)	GLUT-1 positivity (%)	Desmin loss (%)
n	61/88 (69)	54/81 (67)	74/80 (92)
Mean age	57	56	55
Sex	28M, 33F	26M, 28F	34M, 40F
Pleura	48/66 (73)	37/61 (61)	57/60 (95)
Peritoneum	13/20 (65)	15/18 (83)	15/18 (83)
Pericardium	0/1 (0)	1/1 (100)	1/1 (100)
Tunica albuginea	0/1 (0)	1/1 (100)	1/1 (100)
Epithelioid type	50/75 (67)	46/68 (68)	62/67 (93)
Biphasic type	10/12 (83)	8/12 (67)	12/12 (100)
WDPM	1/1 (100)	0/1 (0)	0/1 (0)
Control (n = 35)	0/35 (0)	6/33 (18)	10/34 (29)

TABLE 1 Summary of the immunohistochemical results in biopsy specimens

Abbreviations: BAP1, BRCA1-associated protein-1; F, female; GLUT-1, glucose transporter-1; M, male; WDPM, well-differentiated papillary mesothelioma.

2.2 | IHC evaluation

For BAP1 IHC, cases with complete absence of nuclear signal in mesothelial cells were recorded as positive for BAP1 loss (any immunopositivity was recorded as retained BAP1). As loss or presence of nuclear signals was diffuse, we did not use any cut-off value for scoring. We used background cells, such as inflammatory cells in cytological specimens and fibroblasts/endothelial cells in tissue sections as internal control. For the expression of GLUT-1 and desmin, we evaluated membranous and/or cytoplasmic staining in mesothelial cells. IHC results for GLUT-1 and desmin were assessed semi-quantitatively (negative: no immunoreactivity at all, positive: >1% cells positive). Although we did not use a cut-off level for GLUT-1 and desmin immunopositivity, we arbitrarily made two positives (focal positive: 1%-10% positive, diffuse positive: >10%) to investigate the influence of these cut-off levels on diagnostic accuracy of the test.

2.3 | Statistical analysis

Fisher exact probability test was used to assess the association between variables, including age, sex, tumour histological subtype, tumour location and IHC results. A P value of <0.05 was considered statistically significant. Fisher exact test was calculated using SPSS 13.0.

3 | RESULTS

3.1 | Tissue biopsies

Results of IHC and cohort characteristics are summarised in Table 1. Briefly, BAP1 loss was observed in 61/88 MM cases (69.3%). BAP1 loss was detected more often in women (54%), in the pleura (72.7%), in biphasic histological type (83.3%) and in the inhabitants of erionite

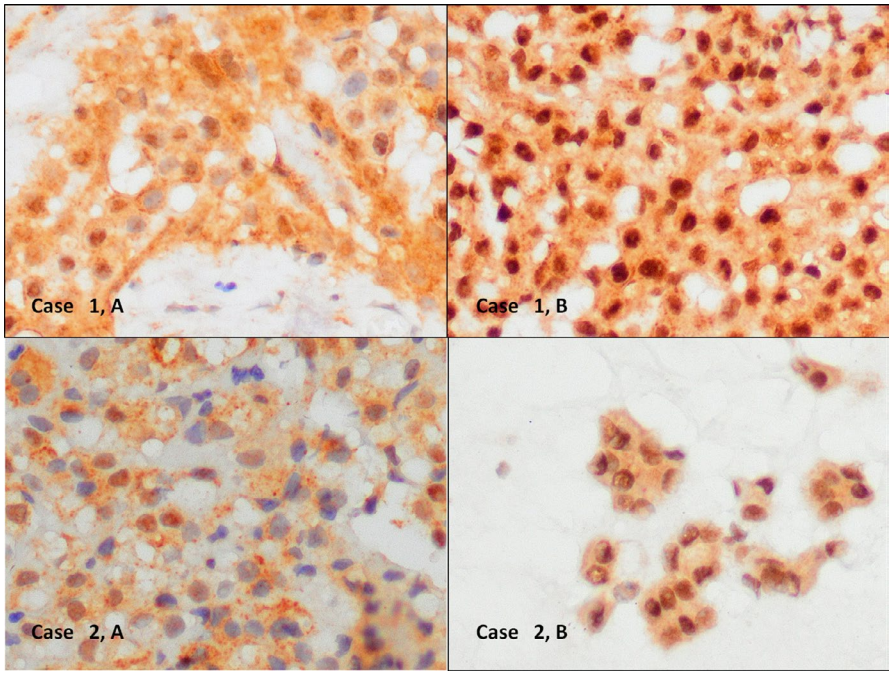

FIGURE 1 Two malignant mesothelioma cases in tissue microarray with presence of both BRCA1-associated protein (BAP1)-positive and -negative cell population (1A and 2A). Both cases are scored as BAP1 positive (eg, BAP1 expression retained) after repeat immunohistochemistry in the whole mount section (1B) or in the cell block (2B; ×400)

villages (75% vs 69% asbestos regions). These findings, however, were not statistically significant. None of the 35 control tissues demonstrated BAP1 loss in mesothelial cells. BAP1 staining, when present, was diffuse with moderate to strong intensity, and allowed scoring easily except in two TMA samples in which we observed both BAP1 negative and weak positive MM cells. Both cases were accepted as BAP1 positive (ie, BAP1 retained) after being confirmed by repeat IHC in whole mount section or in the CB (Figure 1).

GLUT-1 and desmin IHC could be evaluated in 81 and 80 cases, respectively. GLUT-1 and desmin signals, when present, were moderate to strong in intensity, and ranged from 1% to >90% of cell population. Among MM cases, 54/81 (66.7%) were positive and 27/81 (33.3%) were negative for GLUT-1. Of GLUT-1 positive cases, 19 (35.2%) showed focal and 35 (64.8%) showed diffuse immunoreactivity. A focal GLUT-1 positivity was also noted in 6/33 (18.2%) control cases. Of 80 MM cases, we recorded 74 (92.5%) negative and 6 (7.5%) positive (five focal and one diffuse) cases with desmin IHC. Desmin loss was observed in 10/34 control cases. Accordingly, sensitivity of GLUT-1 expression and desmin loss for MM was 66.7% and 92.5%, and specificity was 81.8% and 70.6%, respectively. We did not find any noticeable relation between GLUT-1 or desmin expression and cohort characteristics.

3.2 | Cell blocks and cytospins

As summarised in Table 2, we detected BAP1 loss in 20/30 (66.6%) MM cases from 19 cell blocks and one cytospin preparation. Although observed more frequently in males (60%) and in pleural effusions (75%), this association was not statistically significant. As BAP1 is a nuclear antibody, positive signals could be interpreted easily in most cases. In some MM cases, however, we observed cytoplasmic BAP1 staining, associated with or without a nuclear signal (Figure 2). By contrast, interpretation of nuclear signals for BAP1 was challenging in some CB sections that consisted exclusively, or almost exclusively, of inflammatory cells, particularly macrophages. When CBs contained few scattered benign or malignant mesothelial cells, differentiation from macrophages and, thus, evaluation of BAP1 loss was not easy. In one such case, we could identify mesothelioma cells by their crowded organisation and cellular atypia. In two cases without cytological or architectural atypia, we first confirmed the presence and amount of mesothelial cells, and differentiated them from macrophages by using calretinin (Figure 3). In another case with a faint BAP1 signal, we repeated BAP1 IHC in a later effusion sample from the same patient where we observed BAP1 loss.

We did not observe BAP1 loss in any control cases. In two cases, however, we noted few scattered BAP1 negative cells among BAP1 positive benign mesothelial cell groups. One of those cases had undergone pleural fluid sampling during coronary by-pass surgery, and the other case (Figure 4) was a peritoneal lavage performed during excision of a cystic ovarian neoplasm, which was proved to be a Leydig cell tumour histopathologically. We concluded that BAP1 could rarely present a patchy immunoreactivity (partial loss) in benign elements, such as mesothelial cells or even inflammatory cells, most probably due to technical issues. We further evaluated the performance of BAP1 IHC in seven pre-stained cytological slides (four cytospin preparations and three fine needle aspiration smears), four of which also had CBs. The immunoreactivity of BAP1 on these cases was highly satisfactory both on malignant and benign cellular elements, and correlated perfectly not only with corresponding tissues, but also with four CBs, three of which showed BAP1 loss and one BAP1 immunoreactivity (Figure 5).

TABLE 2 Summary of the immunohistochemical results in cytology samples

	BAP1 loss (%)	GLUT-1 positivity (%)	Desmin loss (%)
n (%)	20/30 (67)	23/25 (92)	16/21 (76)
Mean age	62	57	60
Sex	12M, 8F	12M, 11F	10M, 6F
Pleural fluid	12/16 (75)	12/14 (86)	9/11 (82)
Peritoneal fluid	8/14 (57)	11/11 (100)	7/10 (70)
Control (n = 30)	0/30 (0)	6/30 (20)	0/29 (0)

Abbreviations: BAP1, BRCA1-associated protein-1; GLUT-1, glucose transporter-1.

Among 30 cytology cases, GLUT-1 and desmin IHC could be evaluated in 25 and 21 cases, respectively. We detected GLUT-1 expression in 23 (92%) and desmin loss in 16 (76.2%) of malignant effusions when cut-off value was set to >1%-10% (Table 2). GLUT-1 and desmin expression did not correlate with age, sex or effusion location.

3.3 | Diagnostic performance of IHC markers in tissue biopsies and cytological samples

Diagnostic performance of antibodies, alone and combined, is shown in Table 3. A total of 34 out of 78 (43.6%) biopsies and 15 out of 20 (75%) cytology specimens showed IHC properties favouring mesothelioma with the three antibodies together (eg, presence of BAP1 loss, GLUT-1 positivity and desmin loss; Figure 6). A total of 19/33 (57.6%) benign biopsies and 23/29 (79.3%) benign effusions demonstrated IHC profile in favour of RMP with all three antibodies (eg, BAP1 positivity, GLUT-1 negativity and desmin positivity). Combined use of three markers (eg, presence of any of BAP1 loss, or GLUT-1 expression, or desmin loss) resulted with increased sensitivity (100%), but decreased specificity (79 3%), without a significant influence on diagnostic accuracy. As detailed in Table 4, concordance rate of IHC results between biopsy and CB for BAP1, GLUT-1 and desmin were 100%, 72% and 71%, respectively. Four out of five discordant GLUT-1 cases and four out of four discordant desmin cases showed negative immunoreactivity in the tissues while positive in CBs.

4 | DISCUSSION

Our results were similar to those of previous studies, in that BAP1 loss is frequently seen in MM, and it is limited only to malignancy.

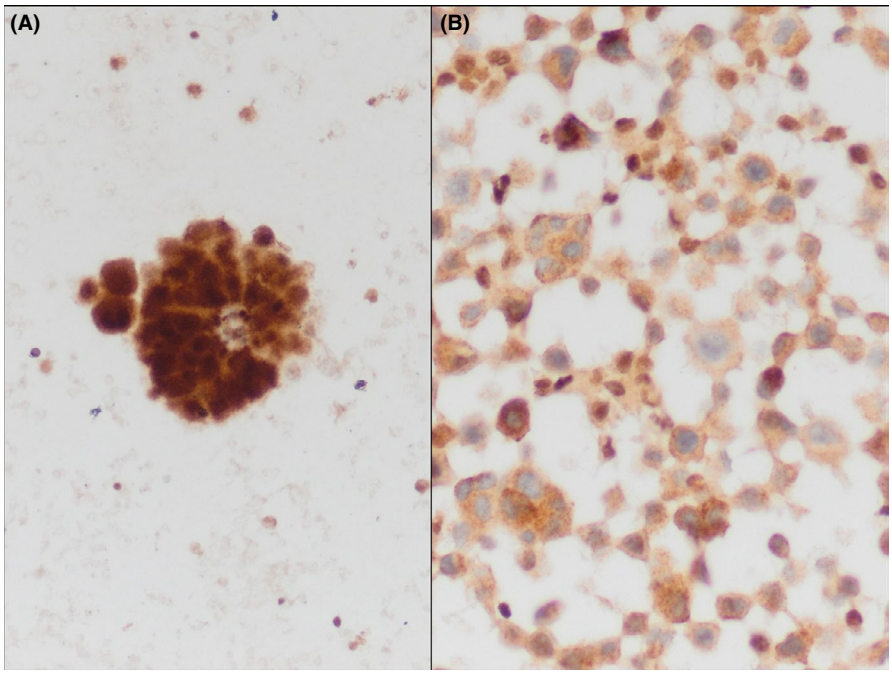

FIGURE 2 Two malignant mesothelioma cases with cytoplasmic BRCA1-associated protein-1 staining which is associated with (A) and without (B) nuclear BRCA1-associated protein-1 signal (×400)

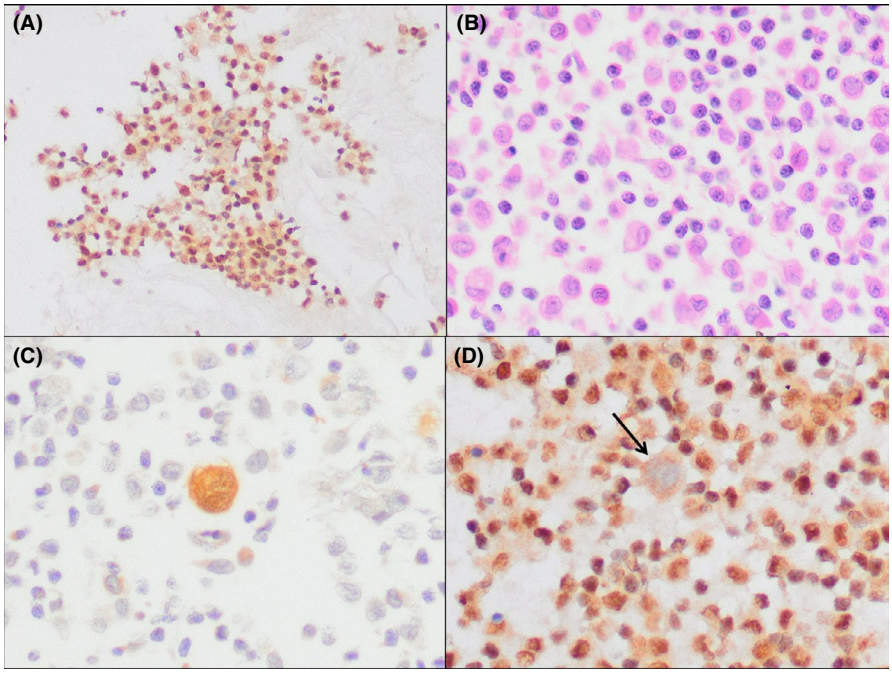

FIGURE 3 Numerous BRCA1-associated protein-1 (BAP1) positive cells (A) are seen in this cell block of a malignant mesothelioma case rich in inflammatory cells (B). There are very few mesothelioma cells, as highlighted by calretinin immunoreactivity (C), and demonstrate loss of BAP1 expression (D, arrow). BAP1 positive cells are background inflammatory cells. Double stain for mesothelial cells and macrophages may be helpful in such cases (A; ×200, B,C,D; ×400)

We observed loss of BAP1 in 69% of MM biopsy specimens and 67% of MM effusions with an excellent (100%) concordance rate, which is in agreement with previous reports.[18,19] The frequency of BAP1 loss varied between 27% and 76% in several studies, and it is reported more commonly in epithelioid subtype and pleural location.[18,20-23] Although we observed a slightly higher frequency in biphasic mesotheliomas (in the epithelioid component) and in pleural tumours, these data and other clinical variables such as possible fibre type (asbestos or erionite), patient age, sex or prognosis were not significantly correlated. However, BAP1 mutation or loss of BAP1 expression has been shown to be associated with better prognosis.[21,24]

We and most previous studies observed 100% specificity for BAP1 IHC in both tissues and cytological samples. Few studies, however, have reported lower rates of specificity, and explained it by factors related to interpretation errors or application of strict cut-off values that can be as high as 50%.[22,25] A cut-off value for BAP1 immunoreactivity has not been applied in most studies, including ours, because a standard value has not yet been defined, and BAP1 signals were of homogeneous quality in most studies, allowing an effortless interpretation. In some biopsy and CB samples, however, we experienced several difficulties, such as the presence of both BAP1 positive and negative mesothelial cells in the same

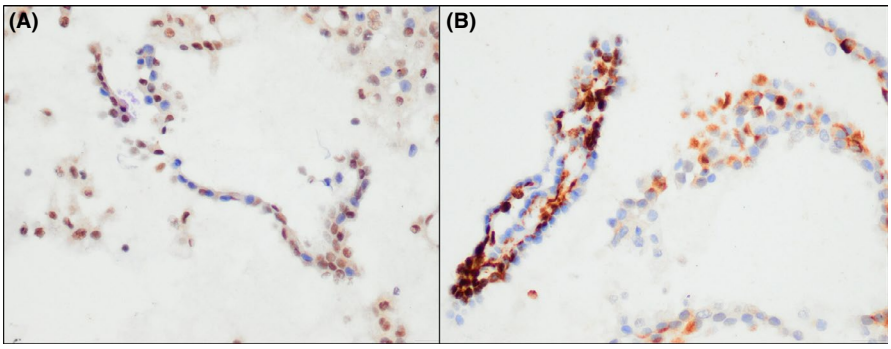

FIGURE 4 In this control cell block, a patchy immunoreactivity (partial loss) with BAP1 (A) and desmin (B) are observed in benign mesothelial cells (×200)

case; weak nuclear signal quality in some cases; occasional presence of associated cytoplasmic staining; and difficulty in discriminating of macrophages from mesothelial cells, particularly in CBs. We had one case from the TMA section that showed weak nuclear BAP1 signal in some mesothelioma cells while it was negative in the others. Corresponding CB of this case was BAP1 positive. Therefore, we prepared a whole mount section from the biopsy and observed that the tumour cells preserved BAP1 expression diffusely. In another MM case, BAP1 immunostaining on CB showed scattered weakly positive mesothelial cells among clearly negative ones. Repeat IHC in a later effusion of this case revealed BAP1 immunonegativity in the tumour. In their study, Cigognetti et al[23] reported two such MM cases containing distinct cell populations regarding BAP1 immunostaining. Presence of such double mesothelial cell population may be a result of intra-tumoural heterogeneity or contaminating normal mesothelial cells.[23,26] Preanalytical issues such as fixation method or IHC procedures may also be factors to explain such a patchy staining pattern. Cases with weak BAP1 signals or presenting both positive and negative cells should be interpreted cautiously and in correlation with the clinical findings. By contrast, granular cytoplasmic staining with BAP1 was not infrequent in our MM cases. When intense, cytoplasmic staining can obscure the true nature of nuclear signal and lead to interpretation errors. Cytoplasmic staining in MM has been reported up to 27% and explained by sequestration of some mutated BAP1 isoforms in the cytoplasm due to prevented nuclear localisation, resulting in loss of nuclear signal.[26,27] Some authors have suggested that cytoplasmic BAP1 staining that is together with nuclear negativity could be associated with better prognosis.[26,28] Righi et al[28] showed that 100% of the MM cases with this type of reactivity were mutated, concluding that it is a reliable predictor of BAP1 mutation.

Macrophages, particularly in effusions, may act as excellent mimickers of mesothelial cells and lead to false positive interpretation of BAP1 when they outnumber mesothelial cells. However, we also observed that some macrophages (and also lymphocytes) did not always retain BAP1 signal, making the evaluation even more complicated. Thus, we suggest using an outer control for BAP1, and specific immunostains, such as CD68, to highlight macrophages. A double immunostaining for BAP1 with a membranous mesothelial marker, such as D2-40, or a cytoplasmic mesothelial marker such as

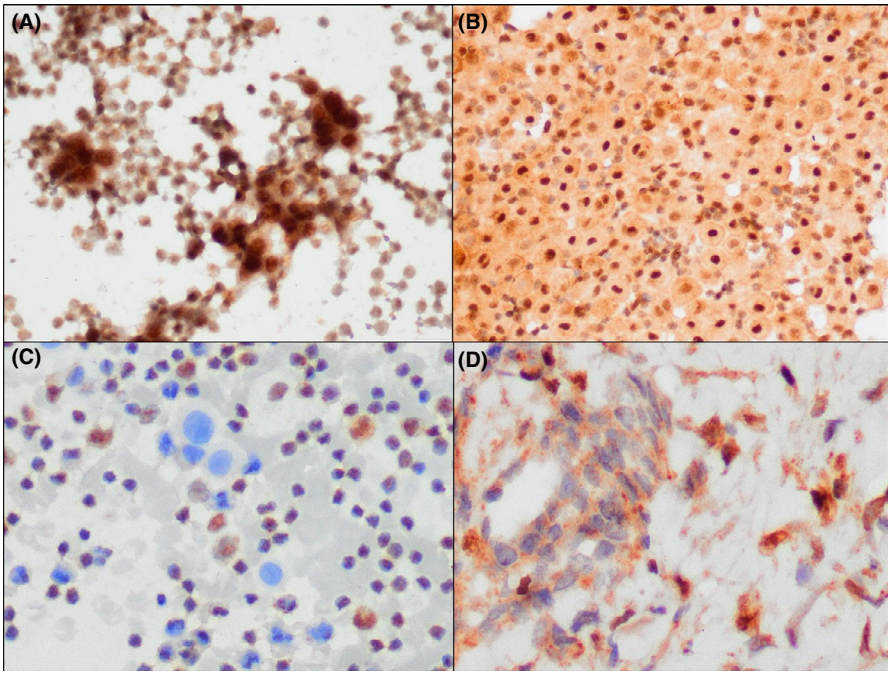

FIGURE 5 Performance of BRCA1-associated protein-1 (BAP1) immunocytochemistry on two pre-stained cytological slides and their corresponding cell blocks (A and B): FNA cytology and its CB with BAP1 signal, ×400; (C and D): cytospin preparation and its CB with BAP1 loss, ×400)

TABLE 3 Immunohistochemical performance of antibodies in biopsy and cytology samples

	BAP1 loss Biopsy/cytology	GLUT1 positivity Biopsy/cytology	Desmin loss Biopsy/cytology	Combined Biopsy/cytology
Sensitivity	69/67	67/92	93/76	100/100
Specificity	100/100	82/80	71/100	58/79
NPV	57/75	50/92	80/85	100/100
PPV	100/100	90/79	88/100	85/77
DA	78/88	71/86	86/90	87/88

Abbreviations: BAP1, BRCA1-associated protein-1; DA, diagnostic accuracy; GLUT-1, glucose transporter-1; NPV, negative predictive value; PPV, positive predictive value.

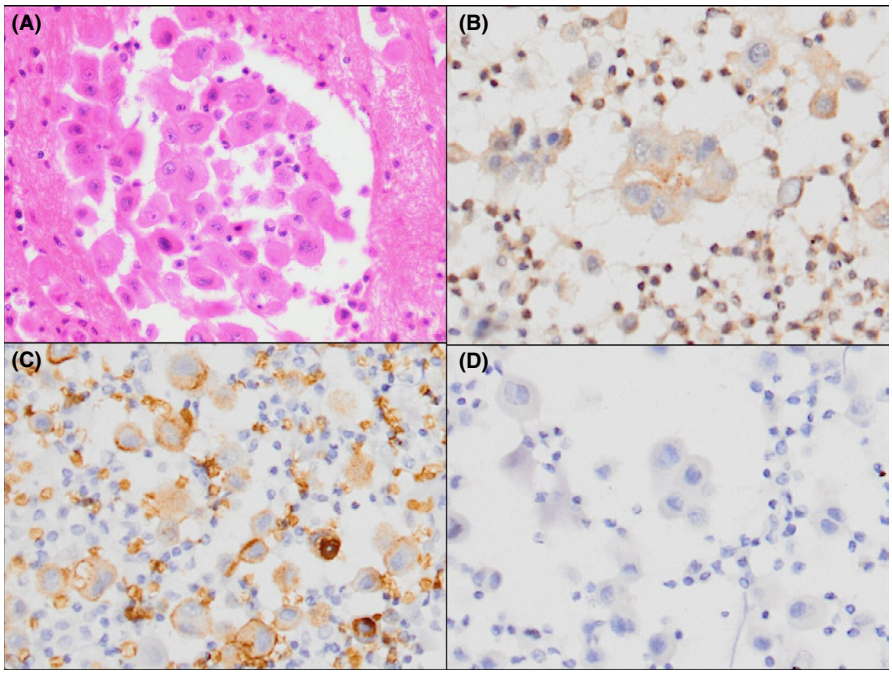

FIGURE 6 A cell block from malignant pleural mesothelioma demonstrating typical IHC features. Neoplastic cells are BRCA1-associated protein-1-negative (B), glucose transporter-1 positive (C) and desmin negative (D; ×400)

CK5/6, may also be very useful to distinguish macrophages from mesothelial cells.

Diagnostic accuracy of GLUT-1 in tissues and effusions has varied from low-to-moderate to 100% in previous reports.[12,16] We identified GLUT-1 immunoreactivity in 67% of biopsies and 92% of CBs (with diagnostic accuracy rates of 71% and 85%, respectively), which was consistent with the average values in the literature. Our study also confirmed that loss of desmin expression is not an infrequent event in MM (92% of biopsies, 76% of effusions) and can be used in the IHC panel with its diagnostic accuracy rates of 86% and 90%, respectively. Although specificity of desmin in CBs was 100%, the relatively lower rate (71%) of concordance between biopsies and CBs raises doubt about its reliability.

Among the three antibodies we studied, GLUT-1 and desmin showed higher rate of variation in the immunoreactivity, which ranged from few to almost all cells. One of the major drawbacks of present study was scoring the cases that contained limited number of staining cells (eg, *focal positive* group in our cohort) for GLUT-1 and desmin. Concordance between biopsy and cytology for GLUT-1 and desmin immunoreactivity was almost identical (72% vs 71%, respectively) and significantly lower than that of BAP1 (100%). It is important to underline the fact that staining patterns of antibodies (membranous, cytoplasmic or nuclear), differences in antibody clones, dilutions, antigen retrieval methods, interpretation of signal quality, as well as the cut-off values applied are potential factors that contribute to various results in the diagnostic performances of the tests and concordance between biopsies and cell blocks.

TABLE 4 Concordance of immunoreactivity in biopsy and cell block samples from mesotheliomas

		Biopsy/cell block				
	n	+/+	−/−	±	−/+	Concordance (%)
BAP1	20	5	15			20/20 (100)
GLUT-1	18	12	1	1	4	13/18 (72)
Desmin	14		10		4	10/14 (71)

Abbreviations: BAP1, BRCA1-associated protein-1; GLUT-1, glucose transporter-1.

When our cases were also analysed for the value of combined IHC for the three antibodies, sensitivity and negative predictive value of the test reached 100% in both tissues and effusions, and specificity decreased to 58% for the tissues and 79% for the cell blocks. We obtained a mild to moderate increase in the overall diagnostic accuracy rate, which is 87% for both sampling methods.

In conclusion, our study demonstrated that BAP1, GLUT-1 and desmin are useful adjuncts in the discrimination between RMP and MM in biopsies and effusions with moderate-to-high sensitivity. A combined IHC panel may improve sensitivity up to 100%, decreasing the specificity. However, BAP1 loss, alone, seems to be evidence in favour of MM with its excellent specificity and can be of greater value when dealing with small biopsies and cytological material. More accurate interpretation of the signals due to nuclear staining characteristics of BAP1 and its 100% concordance rate between all sampling methods seems to be an advantage over GLUT-1 and desmin, which also merit credit by contributing to the sensitivity of the test especially when used combined. However, IHC should be interpreted prudently and always correlated with clinical and radiological features.

ACKNOWLEDGEMENTS

I wish to acknowledge the help and support by Dr Salilh Emri for conceptualisation and resources. I would like to thank Dr Deniz Koksal and Dr Sevinc S. Ulasli providing the clinical data of study cases. I am particularly grateful for the assistance of Dr Ece Ozogul in providing the archival pathological material and constructing tissue microarrays with patience. Finally, I would like to express my very great appreciation to Dr Pinar Firat for her supervision and advices.

AUTHOR CONTRIBUTIONS

Sevgen Önder: Conceptualisation, data curation, investigation, methodology, validation, writing original draft and editing. Ece Ozogul: Data curation, investigation, methodology, validation. Deniz Koksal: Data curation, investigation. Sevinc S. Ulasli: Data curation, investigation. Pinar Firat: Data curation, investigation, methodology, validation, editing, supervision. Salih Emri: Resources, data curation, investigation, supervision. All authors have read and contributed to the final manuscript and confirm that this is an original work that has not been previously published, nor has it been submitted to another journal for simultaneous review. The contents do not constitute an infringement of any copyright laws, nor are they libellous or an invasion of privacy. The authors have no conflicts of interest or other relationships that that might lead to bias. We received no financial support from any outside organisations.

ORCID


Sevgen Önder https://orcid.org/0000-0002-5523-0669

REFERENCES

1. Baris YI, Artvinli M, Sahin AA. Environmental mesothelioma in Turkey. *Ann N Y Acad Sci*. 1979;330:423-432.
2. Emri S, Demir A, Dogan M, et al. Lung diseases due to environmental exposures to erionite and asbestos in Turkey. *Toxicol Lett*. 2002;127(1–3):251-257.
3. Carbone M, Baris YI, Bertino P, et al. Erionite exposure in North Dakota and Turkish villages with mesothelioma. *Proc Natl Acad Sci USA*. 2011;108(33):13618-13623.
4. Roushdy-Hammady I, Siegel J, Emri S, Testa JR, Carbone M. Genetic-susceptibility factor and malignant mesothelioma in the Cappadocian region of Turkey. *Lancet*. 2001;357(9254):444-445.
5. Dogan AU, Baris YI, Dogan M, et al. Genetic predisposition to fiber carcinogenesis causes a mesothelioma epidemic in Turkey. *Cancer Res*. 2006;66(10):5063-5068.
6. Husain AN, Colby T, Ordonez N, et al. Guidelines for pathologic diagnosis of malignant mesothelioma: 2012 update of the consensus statement from the International Mesothelioma Interest Group. *Arch Pathol Lab Med*. 2013;137(5):647-667.
7. Hjerpe A, Ascoli V, Bedrossian CW, et al. Guidelines for the cytopathologic diagnosis of epithelioid and mixed-type malignant mesothelioma. Complementary statement from the International Mesothelioma Interest Group, also endorsed by the International Academy of Cytology and the Papanicolaou Society of Cytopathology. *Acta Cytol*. 2015;59(1):2-16.
8. Wan C, Shen YC, Liu MQ, et al. Diagnostic value of fluorescence in situ hybridization assay in malignant mesothelioma: a meta-analysis. *Asian Pac J Cancer Prev*. 2012;13(9):4745-4749.
9. Ventii KH, Devi NS, Friedrich KL, et al. BRCA1-associated protein-1 is a tumor suppressor that requires deubiquitinating activity and nuclear localization. *Cancer Res*. 2008;68(17):6953-6962.
10. Carbone M, Yang H, Pass HI, Krausz T, Testa JR, Gaudino G. BAP1 and cancer. *Nat Rev Cancer*. 2013;13(3):153-159.
11. Testa JR, Cheung M, Pei J, et al. Germline BAP1 mutations predispose to malignant mesothelioma. *Nat Genet*. 2011;43(10):1022-1025.
12. Kato Y, Tsuta K, Seki K, et al. Immunohistochemical detection of GLUT-1 can discriminate between reactive mesothelium and malignant mesothelioma. *Mod Pathol*. 2007;20(2):215-220.
13. Ikeda K, Tate G, Suzuki T, Kitamura T, Mitsuya T. Diagnostic usefulness of EMA, IMP3, and GLUT-1 for the immunocytochemical distinction of malignant cells from reactive mesothelial cells in effusion cytology using cytospin preparations. *Diagn Cytopathol*. 2011;39(6):395-401.
14. Lee AF, Gown AM, Churg A. IMP3 and GLUT-1 immunohistochemistry for distinguishing benign from malignant mesothelial proliferations. *Am J Surg Pathol*. 2013;37(3):421-426.
15. Attanoos RL, Griffin A, Gibbs AR. The use of immunohistochemistry in distinguishing reactive from neoplastic mesothelium. A novel use for desmin and comparative evaluation with epithelial membrane antigen, p53, platelet-derived growth factor-receptor, P-glycoprotein and Bcl-2. *Histopathology*. 2003;43(3):231-238.
16. Hasteh F, Lin GY, Weidner N, Michael CW. The use of immunohistochemistry to distinguish reactive mesothelial cells from malignant mesothelioma in cytologic effusions. *Cancer Cytopathol*. 2010;118(2):90-96.
17. King J, Thatcher N, Pickering C, Hasleton P. Sensitivity and specificity of immunohistochemical antibodies used to distinguish between benign and malignant pleural disease: a systematic review of published reports. *Histopathology*. 2006;49(6):561-568.
18. Hwang HC, Sheffield BS, Rodriguez S, et al. Utility of BAP1 Immunohistochemistry and p16 (CDKN2A) FISH in the diagnosis of malignant mesothelioma in effusion cytology specimens. *Am J Surg Pathol*. 2016;40(1):120-126.

19. Kinoshita Y, Hida T, Hamasaki M, et al. A combination of MTAP and BAP1 immunohistochemistry in pleural effusion cytology for the diagnosis of mesothelioma. *Cancer Cytopathol*. 2018;126(1):54-63.
20. Sheffield BS, Hwang HC, Lee AF, et al. BAP1 immunohistochemistry and p16 FISH to separate benign from malignant mesothelial proliferations. *Am J Surg Pathol*. 2015;39(7):977-982.
21. Farzin M, Toon CW, Clarkson A, et al. Loss of expression of BAP1 predicts longer survival in mesothelioma. *Pathology*. 2015;47(4):302-307.
22. Andrici J, Sheen A, Sioson L, et al. Loss of expression of BAP1 is a useful adjunct, which strongly supports the diagnosis of mesothelioma in effusion cytology. *Mod Pathol*. 2015;28(10):1360-1368.
23. Cigognetti M, Lonardi S, Fisogni S, et al. BAP1 (BRCA1-associated protein 1) is a highly specific marker for differentiating mesothelioma from reactive mesothelial proliferations. *Mod Pathol*. 2015;28(8):1043-1057.
24. Baumann F, Flores E, Napolitano A, et al. Mesothelioma patients with germline BAP1 mutations have 7-fold improved long-term survival. *Carcinogenesis*. 2015;36(1):76-81.
25. Walts AE, Hiroshima K, McGregor SM, Wu D, Husain AN, Marchevsky AM. BAP1 immunostain and CDKN2A (p16) FISH analysis: clinical applicability for the diagnosis of malignant mesothelioma in effusions. *Diagn Cytopathol*. 2016;44(7):599-606.
26. Nasu M, Emi M, Pastorino S, et al. High incidence of somatic BAP1 alterations in sporadic malignant mesothelioma. *J Thorac Oncol*. 2015;10(4):565-576.
27. Ascoli V, Cozzi I, Vatrano S, et al. Mesothelioma families without inheritance of a BAP1 predisposing mutation. *Cancer Genet*. 2016;209(9):381-387.
28. Righi L, Duregon E, Vatrano S, et al. BRCA1-associated protein 1 (BAP1) immunohistochemical expression as a diagnostic tool in malignant pleural mesothelioma classification: a large retrospective study. *J Thorac Oncol*. 2016;11(11):2006-2017.

How to cite this article: Önder S, Özogul E, Koksal D, Ulasli SS, Firat P, Emri S. Diagnostic value of BRCA1-associated protein-1, glucose transporter-1 and desmin expression in the discrimination between reactive mesothelial proliferation and malignant mesothelioma in tissues and effusions. *Cytopathology*. 2019;30:592-600. https://doi.org/10.1111/cyt.12738