Can BAP1 expression loss in mesothelial cells be an indicator of malignancy?

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Background: Malignant mesothelioma is a highly aggressive tumor that can be confused with a benign mesothelial lesion, especially cytormorphologic lesions. BRCA1-associated protein 1 (BAP1) acts as a tumor suppressor. In this study, we aim to investigate the value of BAP1 staining of malignant mesothelioma cases with expression loss and diagnosis in cell block and biopsy tissue. Methods: Between January 2009 and March 2017, 64 mesotheliomas, 117 reactive mesothelial hyperplasias, and 20 fibrinous pleuritis/pericarditis were diagnosed with morphologic and immunohistochemical findings in our pathology clinic and were included in the study. Formalin-fixed, paraffin-embedded tissues were immunohistochemically examined for BAP1. Inflammatory and stromal cells were used as positive internal controls. BAP1 was assessed for nuclear staining in mesothelial cells. Results: Examinations of the relationship between patient diagnosis and BAP1 biopsy status showed that the BAP1 loss rate (76.6%) was significantly higher in malignant mesothelioma cases than in other benign diseases (0%) (p < .001). Sensitivity and specificity were 76.56% and 100%, respectively, for biopsy tissue from malignant mesothelioma. Sensitivity and specificity were both 100% for BAP1 test on cell block tissue. Furthermore, the consistency between BAP1 cell block and biopsy results was excellent (κ = 0.90) and the correlation was significant (p < .001). Conclusions: This study shows that BAP1 expression loss in both cytology and biopsy tissue in biopsy-confirmed malignant mesothelioma cases is an essential parameter for malignant mesothelioma diagnosis.

Key Words: Asbestosis; BAP1; Immunohistochemistry; Malignant mesothelioma; Pleura

Malignant mesothelioma (MM) is an aggressive tumor originating from serosal surfaces, mostly pleura [1]. Exposure to some carcinogens, especially asbestos, is a strong risk for MM development [2,3]. MM has an inadequate response to treatment because in most patients diagnosis is delayed. However, prognosis is better if recognized earlier. Therefore, it is crucial to diagnose MM early [4-6].

About 80% of MM cases are associated with asbestos exposure. MM incidence is high in Turkey, especially the Cappadocia region, where environmental asbestos is widely distributed [7]. It is thought that 20 years or more of asbestos exposure can increase the risk of MM development.

Malignant mesothelioma, benign mesothelial neoplasia, and reactive mesothelial proliferation are lesions that are difficult to diagnose because they mimic each other cytormorphologically. Although the features supporting malignancy include clear cytological atypia, dense cell clusters, and necrosis, the most reliable diagnostic criterion is the presence of deep-tissue invasion [8]. MM diagnosis in cytolologic materials is even more difficult because invasion is the only reliable standard.

To date, an immunohistochemical marker that can reliably differentiate a reactive process from neoplastic mesothelioma does not exist [9,10]. Various immunohistochemical markers (epithelial membrane antigen, p53, p100, p-glycoprotein, desmin, etc.) are used together to reach a final decision [11-15].

BRCA1-associated protein 1 (BAP1) acts as a tumor suppressor and belongs to the family of high-risk cancer-related genes located at 3p21.1. It is associated with a high-risk cancer syndrome that includes malignancies such as malignant mesothelioma, uveal melanoma, cutaneous melanoma, atypical melanocytic tumor, and renal cell carcinoma (especially clear-cell type). BAP1 expression loss may be an indicator of malignancy in mesotheli-
immunohistochemical staining process, including deparaffini-
sation with xylene, and closure of the sections with lamel.

Inflammatory and stromal cells in the cell block of cytologic
materials and biopsy materials were used as a positive internal
control for BAP1 antibody. Mesothelial cells were evaluated via
nuclear staining with BAP1 antibodies. Detection of nuclear
staining was interpreted as "BAP1 normal expression" (Fig. 1A–D)
and lack of staining as "loss of BAP1 expression" (Fig. 2A–D).
Cytoplasmic staining, observed in some cases, was evaluated as
nonspecific, and we took into account only nuclear staining.

**MATERIALS AND METHODS**

**Case selection**

Sixty-four patients with mesothelioma, 117 patients with re-
active mesothelial hyperplasia, and 20 patients with FP who were
diagnosed at our pathology clinic between January 2009 and
March 2017 were included in the study. Diagnosis of the MM
cases was made according to presence of deep-tissue invasion
composed of mesothelial cells using immunohistochemically
applied calretinin, WT1, and D2.40 stains of biopsy tissue. The
cell blocks obtained from cytological material taken during biop-
sy were evaluated for the presence of mesothelial cells. The cell
blocks, which were also stained with the same immunohisto-
chemical markers, were also analyzed for this study.

We examined slides, paraffin blocks, and report archives of all
the study cases where available. Cell blocks of cytological material
and biopsy tissue were evaluated for suitability for immunohis-
tochemical examination. Both cell blocks and tissue blocks that
had sufficient mesothelial cells (≥ 20 cells) were immunohisto-
chemically stained with BAP1 antibodies. For cases that did
not have cell blocks or that had insufficient mesothelial cells in
cell blocks, only tissue blocks were stained. Two pathologists,
blinded from the diagnosis, evaluated results on light microscopy.

**Immunohistochemical procedure**

For immunohistochemical examination, 4-μm-thick sections
prepared from formalin-fixed and paraffin-embedded tissues
were used. Tissue sections were taken into electrostatically charged
slides (Isotherm) and dried at 70°C for at least 1 hour. The whole
immunohistochemical staining process, including deparaffiniza-
tion and antigen release, was performed on a fully automated
immunohistochemistry staining device (Ventana BenchMark
XT, Ventana Medical Systems, Tucson, AZ, USA). We used a
ready-made kit that is biotin-free, based on an horseradish per-
oxidase (HRP) multimer, and contains hydrogen peroxide sub-
strate and 3,3’-diaminobenzidine tetrahydrochloride (DAB)
chromogen (Catalog number 760-500, ultraView Universal DAB
Detection Kit, Ventana Medical Systems) for this process. BAP1
antibody (1:100, Thermo Fisher Scientific, Rockford, IL, USA)
was administered. Contrast staining with hematoxylin and blu-
ing solution was performed with a staining device; the proce-
dure was completed manually with dehydration, transparency
with xylene, and closure of the sections with lamel.

**RESULTS**

Of 64 MM cases, 40 (62.5%) were male and 24 (37.5%) were
female. The median age at diagnosis was 61 (± 12.26) years, and
56.3% of the patients were between 40–64 years old. While 57
of the 64 cases (89.1%) originated in the pleura, six (9.4%) were
from the peritoneum and one (1.5%) from the pericardium. In
histological subtypes, 54 cases (84.4%) were epithelioid type,
five cases (5.2%) sarcomatoid type, and 5 cases (5.2%) biphasic
type. Asbestos exposure was detected in 35.9% of our MM cases.

Seventy-four of 117 RMH cases (63.2%) and 15 of 20 FP cases
(75%) were male, with median ages of 45 (± 19.36) years and
59.5 (± 16.07) years, respectively. Of the 137 non-MM cases
(117 RMH and 20 FP), 103 biopsy specimens (75.1%) were
taken from the pleura, 31 (22.6%) from the peritoneum, and 3
According to biopsy status, the BAP1 expression loss rate (76.6%) in patients diagnosed with MM was significantly higher than among other benign diseases (0%; p < .001). When MM cases were evaluated by histological subtype, the BAP1 expression loss rate was 81.5% in epithelioid-type MM cases, whereas no BAP1 expression loss was observed among sarcomatoid-type MM cases. BAP1 expression loss was observed in all five biphasic cases, but all expression loss was found in the epithelioid components, and no loss was found in the sarcomatoid components (Table 1). For MM diagnosis, the sensitivity and specificity of BAP1 expression loss in tissue biopsies were 76.56% and 100%, respectively.

Only the epithelioid histological subtype of malignant mesothelioma cases had sufficient cell block material. According to cell block status, expression loss was observed in all malignant mesothelioma cases (n = 11), while there was no expression loss in any of the benign processes (8 RMH and 1 FP cases). The sensitivity and specificity of the BAP1 test were both 100% for cell block (Table 2).

When the consistency between cell block and tissue biopsy results for BAP1 expression status was examined, a high consistency (κ = 0.90) and significant correlation were obtained (p < .001). Accordingly, 10 of the 11 patients (90.9%) who were found to have BAP1 expression loss in cell block also showed expression loss in biopsy, while all nine patients (100%) who did not show expression loss in cell block also showed no loss in biopsy (Table 3).

MM cases were evaluated in terms of BAP1 expression loss according to age, gender, asbestos exposure, additional cancer, and localization, and no significant differences were found (all p > .05) (Table 4).
DISCUSSION

Due to high tumor aggressiveness, distinguishing MM cases from reactive processes and diagnosing accurately as early as possible is of great importance [4–6]. However, the only reliable criterion for histopathological diagnosis is detection of fatty tissue invasion [8]. Unfortunately, these criteria cannot be evaluated in cytological materials. For this reason, numerous studies have been conducted to find an immunohistochemical marker that might aid MM diagnosis or even offer a definitive diagnosis.

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**Fig. 2.** BRCA1-associated protein 1 (BAP1) immunohistochemistry results in malignant mesothelioma. Biopsy material with hematoxylin and eosin staining (A) and loss of BAP1 expression (B) with inflammatory cells as a positive internal control in epithelioid mesothelioma. Cell block material with BAP1 staining (C). BAP1 normal expression in sarcomatoid mesothelioma (D).

**Table 1.** BAP1 expression status according to biopsy diagnosis

| Histologic type (n=201) | Loss of BAP1 expression (n=49) | BAP1 normal expression (n=152) | p-value |
|------------------------|-------------------------------|-------------------------------|---------|
| Malignant mesothelioma  | 49 (76.6)                     | 15 (23.4)                     | <.001   |
| Reactive mesothelial hyperplasia | 0                          | 117 (100)                    |         |
| Fibrinous pleuritis/Pericarditis | 0                          | 20 (100)                     |         |

**Table 2.** BAP1 expression status according to cell block diagnosis

| Histologic subtype of MM (n=11) | Loss of BAP1 expression (n=11) | BAP1 normal expression (n=9) | p-value |
|-------------------------------|-------------------------------|-------------------------------|---------|
| Epithelioid type               | 11 (100)                      | 0                             | <.001   |
| Sarcomatoid type               | 0                             | 8 (100)                       |         |
| Biphasic type                  | 0                             | 1 (100)                       |         |

BAP1, BRCA1-associated protein 1; MM, malignant mesothelioma.

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Table 3. Consistency between BAP1 expression status in cell block and biopsy

| BAP1 expression status in cell block | BAP1 expression status in biopsy | Total |
|-------------------------------------|---------------------------------|-------|
| Loss of BAP1 expression             | 10 (60.0)                       | 11 (100) |
| BAP1 normal expression              | 0                               | 9 (100)  |
| Total                               | 10 (60.0)                       | 20 (100) |

Values are presented as number (%).

Table 4. BAP1 expression status according to age, sex, asbestos exposure, neoplastic transformation, and localization in MM cases

| Age (yr) | Loss of BAP1 expression | BAP1 normal expression | p-value |
|----------|-------------------------|------------------------|---------|
| 60.0 (49.0–68.0) | 62.0 (58.0–75.0) | .084 |
| Sex       | Male                    | Female                 |         |
| Male      | 29 (72.5)               | 4 (16.7)               | .322    |
| Female    | 20 (83.3)               | 17 (73.9)              | .708    |
| Asbestos exposure | |         |         |
| No        | 32 (78.0)               | 2 (100)                | .427    |
| Yes       | 17 (73.9)               | 6 (26.1)               | .545    |
| Neoplastic transformation | |         |         |
| No        | 47 (75.8)               | 15 (24.2)              |         |
| Yes       | 2 (100)                 | 0                      |         |
| Localisation | |         |         |
| Pleura     | 43 (75.4)               | 14 (24.6)              |         |
| Periton and paricard | 2 (100) | 0 |         |

Values are presented as median (percentile 25–percentile 75) or number (%).

Various studies have demonstrated that immunohistochemical evidence of BAP1 expression loss in mesothelial cells may be a clue to malignancy. Cozzi et al. [18] reported that BAP1 expression loss in cell blocks obtained from effusions had a sensitivity of 76% for MM diagnosis, and that BAP1 expression loss in biopsy samples had 48.7% sensitivity. Meanwhile, Cigognetti et al. [19] and Walts et al. [20] estimated 100% specificity for BAP1 expression loss. In our study, we estimated 76% sensitivity and 100% specificity from biopsy tissue, but 100% sensitivity and specificity from cell blocks obtained from effusion.

Cozzi et al. [18], who investigated the role of BAP1 expression loss in effusion diagnosis for MM, found that BAP1 expression loss was 76.5% prevalent in mesothelioma cases, while it was 11.7% in mesothelial hyperplasia cases. In the same study, looking at biopsy diagnosis, BAP1 expression loss was 47.5% prevalent in patients diagnosed with mesothelioma, but no BAP1 expression loss was observed in any cases diagnosed with mesothelial hyperplasia. In our study, BAP1 expression loss was 76.6% prevalent in biopsy materials and 100% in cell blocks. Furthermore, no BAP1 expression loss was observed in either cell block or biopsy materials in our mesothelial hyperplasia cases.

There are some differences between MM subtypes in terms of nuclear BAP1 expression loss. Wu et al. [21] grouped 38 pleural MM cases as 29 biphasic and nine epithelioid types and then compared their BAP1 expression loss status and subtypes. In this study, we observed BAP1 expression loss in five of eight epithelioid-type MM cases (62.5%), while also observing loss in both sarcomatoid and epithelioid components in five of 13 biphasic-type MM cases (38.5%). Shinozaki-Ushiku et al. [17] studied 32 MM cases and found BAP1 expression loss in 53% (17/32), among which 14 were epithelioid and 3 were biphasic. Furthermore, two biphasic-type cases showed expression loss in both epithelioid and sarcomatoid components, while only 1 showed BAP1 expression persistence in the sarcomatoid component. In our study, BAP1 expression loss was observed in 81.5% of 54 epithelioid-type MM cases, whereas it was observed in all five biphasic-type MM cases (100%), all of which were detected in the epithelioid component. Moreover, no loss of BAP1 expression was observed in any of our five sarcomatoid-type MM cases.

Massive and recurrent effusions occur in the early stages of MM. Therefore, initial diagnosis is mostly based on cytological samples, especially in the epithelioid type [22]. However, in some cases, effusion tissue alone is not sufficient to diagnose MM. In such cases, diagnosis requires biopsy tissue from the patients. Although the diagnostic power of biopsies have been demonstrated by numerous studies, less invasive methods are preferred by both patients and surgeons.

Pulford et al. [14] included both cytological and biopsy materials of 83 MM cases and 18 cases with malignant pleural effusion due to metastatic adenocarcinoma. In addition to the diagnostic value and prognostic significance of BAP1 expression loss, they also measured the agreement of cytological and biopsy materials with the Kappa test. Pulford et al. [14] reported that BAP1 expression loss was observed in 59% of MM cases and the Kappa value was 0.85. Similarly, we applied BAP1 antibodies to the biopsies of cases with cytological samples and we calculated agreement with the Kappa test. Accordingly, the agreement between cytological and histological specimens for BAP1 expression loss was excellent (κ = 0.90), and the correlation was significant (p < .001).

Environmental exposure to asbestos, especially for regions with abundant white soil, like some provinces of Turkey, is of great importance in MM development. Onder et al. [23] evaluated 88 MM cases from central Ankara, which is the nearest city to Cappadocia; they reported that 95% of the cases had been exposed
to asbestos and no significant relationship was found between asbestos exposure and BAP1 expression loss. In our study, only 35.9% of MM cases were exposed to asbestos. We attribute this difference to the fact that the city where we work is far from Cappadocia, and we could not find as significant a relationship as Onder et al. [23] did.

There were some limitations to our work. Primarily, because of the retrospective nature of our study, it was not possible to obtain all of the cytological specimens from the biopsy materials analyzed by immunohistochemistry for BAP1 expression.

In conclusion, BAP1 expression loss was found in all the cytological materials of the cases whose diagnoses were confirmed by tissue biopsy, and the results were replicated for each case when BAP1 antibodies were applied to biopsy materials. This suggests that BAP1 expression loss is a robust criterion in achieving MM diagnosis from cytological materials. Effusion in the serosal cavity is a common early finding for MM. Furthermore, because cytologic examination is less invasive and easy to perform, it is likely to be the preferred diagnostic method, especially given the results reported here. In patients with only serous effusion and no radiological suspicion of MM but who show evidence of BAP1 expression loss by immunohistochemical analysis of cell blocks, we suggest that invasive MM may develop in the near future and, thus, these patients need strict clinical follow-up.

Ethics Statement
Approval was obtained from the Ethics Committee on May 30, 2017 (protocol number: 2017/514/108/8). Informed consent was obtained from all participants included in the study.

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Conflicts of Interest
The authors declare that they have no potential conflicts of interest.

Funding Statement
No funding to declare.

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