Well-differentiated papillary mesothelioma: clustering in a Portuguese family with a germline BAP1 mutation

C. Ribeiro¹, S. Campelos², C. S. Moura³,4, J. C. Machado⁴,5, A. Justino⁶ & B. Parente¹

Departments of ¹Pulmonology; ²Pathology, Centro Hospitalar de Vila Nova de Gaia/Espinho, Vila Nova de Gaia; ³Pathology Department, Hospital de São João, Porto; ⁴Faculty of Medicine, University of Porto, Porto; ⁵IPATIMUP—Institute of Molecular Pathology and Immunology, University of Porto, Porto, Portugal

Background: Well-differentiated papillary mesothelioma (WDPM) is a rare variant of epithelioid mesothelioma and is considered to be associated with good prognosis due to its clinically indolent behavior and long survival. Most reported cases involve the peritoneum of women at reproductive age with no history of exposure to asbestos, with pleural involvement being less common. The optimal management, including the role of chemotherapy in the treatment of WDPM, remains unsettled.

Patients and methods: The authors describe two cases of WDPM in women of the same family (siblings); the elder with WDPM of the pleura and peritoneum with a 12-year survival period and the younger with a WDPM of the peritoneum diagnosed in 2011 and uveal melanoma diagnosed in 2012. Neither patient had any known exposure to asbestos fibers or any other mineral carcinogens.

Results: After the concurrent diagnosis of WDPM and uveal melanoma, genetic diagnosis was carried out taking into consideration that these two malignancies were recently associated with hereditary BAP1 gene mutations and it was positive for both the patients.

Conclusions: To our knowledge, this is the first description of WDPM in two siblings who also presented with a germline BAP1 mutation. This article provides evidence of the wide clinical spectrum of cancer susceptibility associated with a BAP1 germline mutation.

Key words: BAP1, familiar, mutation, well-differentiated papillary mesothelioma

Introduction

Well-differentiated papillary mesothelioma (WDPM) is a rare subtype of epithelioid mesothelioma, most commonly involving the peritoneum of women usually in their fourth or fifth decade of life [1]. It was originally accepted that asbestos exposure was not relevant to the development of this entity (most importantly in the peritoneum), but more recent series have described exposure to asbestos in some patients [2, 3].

WDPM was generally considered of low malignant potential but requiring long-term surveillance and within a clinicohistological spectrum of papillary peritoneal tumors in women ranging from mesothelial hyperplasia to papillary carcinoma [1, 4]. Recent studies have purported a variable, rather than an entirely benign, prognosis [1]. WDPM cases have been reported in both men and women involving the peritoneum, pleura and tunica vaginalis [4]. Occasionally, WDPM may involve two cavitary surfaces simultaneously, most often the pleural and peritoneal surfaces [5, 6].

To the best of our knowledge, around 60 cases of WDPM of the peritoneum and 40 cases of WDPM of the pleura have been described in the literature [1, 2].

WDPM usually presents with nonspecific clinical and radiological features, but it displays a characteristic histological pattern with diffuse formation of papillae with myxoid fibrovascular cores lined by relatively bland mesothelial cells. Basal vacuoles may be present in the lining cells. Nucleoli are inconspicuous and mitotic figures absent or rare. Invasion of the submesothelial layer is absent or minimal [7]. Mesothelial immunocytochemical markers are positive.

The optimal management remains unknown as there is no clear proof whether specific treatment such as surgery, radiotherapy or chemotherapy may alter the natural history of this disease [7].

For many years, it has been hypothesized that genetic factors may also play a role in the pathogenesis of mesothelioma. This is mostly because some individuals develop mesothelioma following exposure to small amounts of asbestos, while others exposed to high amounts do not. There were also reports on mesothelioma clustering in families in which up to 50% of the members developed mesothelioma, usually following a documented exposure to asbestos or erionite [8].
Recently, it has been demonstrated the existence of a germline BAP1 mutation predisposing to the occurrence of mesothelioma, uveal melanoma, meningioma, lung adenocarcinoma and possibly other cancer types [9–16]. It has been hypothesized that BAP1 germline mutations confer increased susceptibility to cancer and specifically to mesothelioma when there is exposure to asbestos. Nevertheless, the possibility that BAP1 mutations alone may be sufficient to cause mesothelioma cannot be entirely ruled out [9].

Cases

case I

The patient is a 56-year-old female, nonsmoker, worked as a manager in a textile and shoes factory, without any known history of asbestos exposure. She had a family history of cancer—her father and uncle died with pancreas cancer and other uncle died of leukemia.

She was admitted in a Surgery Ward for investigation of abdominal pain and ascites with unknown etiology in September 2011. She had a history of corneal ulcers and a prior bilateral cataract surgery, without any other known pathology and no regular medication (she had done hormonal substitution therapy until some months before the admission).

At the time of admission, she presented with abdominal pain and an increasing abdominal diameter in the previous month. The thoracic and abdominal computer tomography revealed gastric, duodenal and jejunal wall thickening, moderate ascitis, marked thickening of the peritoneal and mesenteric fat with some areas with a nodular pattern. There was no evidence of abdominal lymph node enlargement, pleural or pericardic effusion.

She underwent upper and lower gastrointestinal (GI) endoscopy that showed chronic gastritis without any evidence of malignancy and her gynecological examination was normal (as well as her cervical cytology).

A diagnostic laparoscopy was carried out showing ascitis and multiple nodular implants that were biopsied. The analysis of peritoneal fluid revealed mesothelial cells with a slight atypia and some small groups with papillary configuration; there was no evidence of epithelial cells with malignancy. The histology of the peritoneal surface revealed a papillary neoplasia consisting of fibrovascular cores covered by a single layer of bland cubic cells. Mitotic figures were absent. The immunocytochemistry was positive for pan-cytokeratin antibodies (AE1/AE3), cytokeratin 5 and 6 (CK5/6) and calretinin. The morphological and immunocytochemical features along with the lack of invasion led to the diagnosis of WDPM (Figure 1).

The patient had a normal complete blood cell analysis and the biochemistry showed elevated alkaline phosphatase [115 IU/l (35–104)], gamma-glutamyl transferase [138 IU/l (5–61)], Ca 125 [37.2 U/ml (0–35)] with a normal CEA [0.5 ng/ml (0–4.3)].

The positron emission tomography/computed tomography (CT) revealed diffuse hypermetabolic involvement of the mesenteric fat thickening without any other avid foci.

She started chemotherapy with cisplatinum and pemetrexed in November 2011 and completed four cycles of chemotherapy with full regression of the ascitis and a marked reduction in the peritoneal wall fat thickening in the control CT (Figure 2). After the chemotherapy regimen, she presented with decreased left eye visual acuity and was evaluated for suspected metastasis. The physical examination revealed an intraocular mass in the posterior segment. The orbital magnetic resonance imaging showed a well-defined lesion with 1.5 cm arising from the optic nerve into the vitreus humor with a high protein content. The aspirative cytology was nondiagnostic and an eye enucleation was carried out to differentiate metastasis from uveal melanoma. The histological examination showed neoplastic proliferation of spindle and epithelioid cells with moderate pleomorphism, high nuclear/cytoplasm ratio, vesicular chromatin, melanic pigment in the cytoplasm and atypical mitotic figures without optic nerve invasion which led to the diagnosis of uveal melanoma (Figure 2). She has maintained clinical remission in the follow-up period (supplementary Figure S1, available at *Annals of Oncology* online).

![Figure 1. Mesothelial cells with a cubic shape and without significant atypia (HE, 100×).](image1.jpg)

![Figure 2. Malignant melanoma of choroid (HE, 40×).](image2.jpg)
case II

The patient is a female, 44-year-old at the time of diagnosis, nonsmoker, history teacher, without any known history of asbestos exposure. She is the older sister of the first case patient and has a history of non-insulin dependent diabetes. In 1999, she was diagnosed with a malignant pleural and peritoneal epithelioid mesothelioma in a Portuguese University Hospital, and it was confirmed by an American reference center through peritoneum and pleural biopsies. At that time, she was enrolled in a phase III study of cisplatinum and pemetrexed for the treatment of mesothelioma in the United States and then transferred to our Unit in 2004. She also underwent talc pleurodesis.

She was subjected to multiple chemotherapy regimens until 2005 when she presented stable disease and has been in vigilance ever since.

That patient has a 12-year survival period, which is highly unusual in malignant mesothelioma.

In 2011 after her sister was diagnosed with WDPM, and taking into account the insidious evolution of her disease, a revision of the pathological specimens was carried out.

The revision of the peritoneal biopsies showed a papillary pattern malignancy with myxoid spindles covered by mesothelial cells without significant atypia and lack of invasion (supplementary Figure S2, available at Annals of Oncology online and Figure 3).

After this revision, the diagnosis of WDPM was assumed.

Both patients’ mother died in 1997 while being investigated for ascitis. She was a 72-year-old retired medical doctor who developed ascites and peritoneal thickening without retroperitoneal lymph node enlargement and the pathological report of a diagnostic thoracectomy was compatible with epithelioid peritoneal mesothelioma.

The patient’s medical condition deteriorated rapidly and the patient did not receive any specific therapy. The family pedigree is described in supplementary Figure S3, available at Annals of Oncology online.

Following the recent discovery of the association between germline BAP1 mutations and the occurrence of mesothelioma and uveal melanoma, we decided to collect blood samples from both patients to screen for BAP1 mutations. An informed consent was obtained from both patients before the genetic testing, and this examination was approved by the Hospital Ethics Committee. Constitutional genomic DNA was extracted from blood leucocytes using the MagNA Pure LC DNA Isolation kit-Large Volume (Roche). DNA concentration and purity were assessed using a NanoDrop 2000c spectrophotometer (Thermo SCIENTIFIC). Primer pairs were designed to amplify the 17 coding exons and intron–exon boundaries of BAP1 using the ExonPrimer (www.ihg.gsfs.de/ihg/ExonPrimer.html) web-based tool. PCRs were carried out with touchdown PCR on MyCycler Thermal Cycler (BIORAD) following the QIAGEN Multiplex PCR Handbook 10/2010 (QIAGEN) specifications. Oligonucleotide primer sequences and PCR conditions are available upon request. The PCR products were enzymatically purified using FastAP Thermosensitive Alkaline Phosphatase and Exonuclease I (Fermentas), sequenced with a BigDye Terminator v3.1 Cycle Sequencing kit (Applied Biosystems) and run on the ABI PRISM 3130 x l Genetic Analyzer (Applied Biosystems) according to the manufacturer’s instructions. Variant analysis was carried out with the software Mutation Surveyor 3.24 (Softgenetics). The mutation nomenclature used in this work follows the guidelines indicated by Human Genome Variation Society [17]. DNA mutation numbering is based on the cDNA sequence with position +1 corresponding to the A of the ATG translation initiation codon in the reference sequence (GenBank accession code for BAP1: NM_004656). Mutation descriptions on the protein level consider the initiator methionine as codon 1 and have been checked using the Mutalyzer program (www.LOVLD.nl/mutalyzer/). The genetic analysis revealed that both patients had a frameshift mutation in exon 9 of the BAP1 gene (c.758_759insA; p.Gln253fsX31) (supplementary Figure S3, available at Annals of Oncology online). Each mutation was confirmed by re-amplification of a second PCR product and re-sequencing (supplementary Figure S4, available at Annals of Oncology online).

discussion

Our article describes a family clustering of patients diagnosed with WDPM who also present germline BAP1 mutations.

As previously discussed, WDPM is a rare entity and because of that there is still a high degree of uncertainty of associated risk factors, clinical course and best therapy regimens. While much scientific research has been done on asbestos-related malignant mesothelioma the rarity of WDPM coupled with its good prognosis relegated its research to case reports and reviews by medical oncologists concentrating in the area of diagnosis, prognosis and treatment options.

To the extent of our knowledge, there is no previous description of a clustering of patients with this disease (especially in the first degree relatives such as siblings). Predisposing genetic factors to this condition have also not been described—and this rare type of mesothelioma has a

Figure 3. Mesothelial cells with a cubic shape and without significant atypia (HE, 100×).
differentiating characteristic of being less associated with asbestos exposure (or other mineral carcinogens).

We have done an extensive review of the literature regarding WDPM as well as on the recent articles proving a connection between \textit{BAP1} mutations and the development of mesothelioma (and uveal melanoma). Previous studies have reported frequent somatic mutations of \textit{BAP1} in metastasizing uveal melanomas, with one case having a germline mutation [12]. An association between uveal melanoma, breast and ovarian cancers has been proposed [13]. A paper by Bott et al. [14] reported somatic \textit{BAP1} mutations in 23% of sporadic mesotheliomas.

A recent article studied members of American families that experienced an extremely high incidence of mesothelioma, in spite of very modest exposure to asbestos, and found the presence of germline \textit{BAP1} mutations [9]. This was the first gene reported to modulate mineral fiber-associated carcinogenesis. Furthermore, it was proved that \textit{BAP1} mutations are associated with a hereditary cancer syndrome that predisposes to mesothelioma, uveal melanoma and potentially other cancers. This article hypothesized that when individuals with \textit{BAP1} mutations are exposed to asbestos, mesothelioma predominates. Alternatively, \textit{BAP1} mutation alone may be sufficient to cause mesothelioma [9].

Our study raises some important unanswered issues. First, may the germline \textit{BAP1} mutation predispose to different types of mesothelioma, including the rare and potentially less aggressive WDPM? Is it possible that this type of mesothelioma may appear in patients with this mutation who are not exposed to asbestos? Is the gender an important factor in the histology type of mesothelioma in patients with this mutation?

Second, both patients have children and one of the patients (case I) has a daughter. Should this generation receive genetic testing and counseling and regular follow-up as this mutation predisposes to different types of cancer?

Third, this family has a high burden of malignant diseases. Is it possible that this high prevalence may be related to this mutation that has just now been discovered to be associated with mesothelioma?

\textbf{conclusions}

WDPM is a rare disorder with an indolent course and little is known regarding its genetic or environmental exposure risk factors or best course of treatment. It is thought that asbestos and other mineral carcinogens, which play a very important role in the carcinogenesis of other mesothelioma histological subtypes, may be less important in this condition.

The discovery of germline \textit{BAP1} mutations has brought a new insight into the hypothesis that genetic factors play a role in the carcinogenesis of mesothelioma and our description is consistent with this hypothesis.

To our knowledge, this is the first description of a familiar connection between two patients with WDPM. This is also the first description of a connection between a germline \textit{BAP1} gene mutation and WDPM. This article provides evidence of the wide clinical spectrum of cancer susceptibility associated with \textit{BAP1} germline mutations.

We hope that our study helps to raise awareness of this rare condition and promote further study of its risk factors, prevention and best course of treatment.

\textbf{disclosure}

The authors have declared no conflicts of interest.

\textbf{references}