Type II Pleuropulmonary Blastoma in a 4 Month Old Infant with Negative Dicer1 Mutation on Next Generation Sequencing

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ABSTRACT
Introduction: Pleuropulmonary blastoma (PPB) is a rare, but aggressive tumor in the pediatric population. PPB is a dysontogenetic neoplasm of childhood that involves the lungs and/or pleura. Young relatives of children with PPB have an increased incidence of neoplasias and dysplasias. According to tumor tissue histopathology, PPB evolves from a cystic to solid state over time. PPBs can be sub-classified as type I (purely cystic), type II (having both cystic and solid elements), and type III (completely solid). Type II and type III tumors may be associated with metastasis, with the brain being the most common metastatic site. Due to the primitive nature of cells in the tumor mass, PPBs are very aggressive tumors that are resistant to therapy. The prognosis depends on the histopathology content and tumor type. Respiratory problems are the main complaint and diagnosis can be made only after additional examinations. Genetic relations through family members are associated with mutations in the DICER1 gene; between 60-80% of patients with PPBs are positive for DICER1 mutations. Mosaicism has also been reported. Aim: The aim was to present a case of a 4 month-old infant with type II PPB, who had a negative result for DICER1 mutation in next generation sequencing. To detail the clinical presentation of this patient, we present radiographic and ultrasound findings and results of histopathological analysis, as well as genetic and scintigraphic findings and chemotherapy treatment. Case report: Here we describe the genetic analysis of a patient with PPB who was negative for mutations in DICER1 and who had no relatives with disease. This patient underwent radical resection of the tumor and began therapy, but subsequently died after developing leukopenia and sepsis. Conclusion: This case provides an example of a patient with PPB who was negative for DICER1 mutation upon genetic analysis and emphasizes the potential for disease that does not involve mutation of this gene.

Keywords: Pleuropulmonary blastoma, pediatric malignancy, pulmonary tumors, DICER1 gene, Next Generation Sequencing.

1. INTRODUCTION
Cancer is a leading non-communicable disease that causes death worldwide (1). Pleuropulmonary blastoma (PPB) is a rare malignant embryonic tumor of the lungs that grows during organ development and is diagnosed in the pediatric population.

Fortunately, PPB is rare, but for the 50-60 children and their families around the world who are affected annually, the diagnosis can be devastating. Most patients with PPB develop tumors in the first five years of life (2). The majority of children who have the earliest form of the disease (type I PPB) can be cured with surgery and, in some cases, accompanying chemotherapy. Children with later forms of PPB (type II or type III) often have a much more difficult disease course. Type I PPB occurs more frequently in infants (9 to 10 months), in contrast to type II and type III that are more often found in older patients (median age at diagnosis 34 and 44 months, respectively).

The formation of PPB tumors can begin with multiple air-filled cysts that involve primitive mesenchymal cells (type I PPB). In subsequent stages, mesenchymal cells overgrow the delicate septa to produce a sarcomatous neoplasm that is either cystic and solid or exclusively solid (3, 4). Type I PPB is characteristically multicystic. In type II disease nodules may be present within the cystic growth and Type III involves solid masses.
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Among patients diagnosed with PPB, type I has the most favorable prognosis, with 85-90% overall survival and, as mentioned above, is typically detected during infancy (9-10 months). Meanwhile, type II and type III PPB is diagnosed later (34-36 and 42 months, respectively) and these tumors are far more aggressive (6).

The preeminent site for metastatic spread of PPB is usually the brain. Metastatic deposits have a more simple histological appearance compared to the diverse morphology present in the primary tumor. These metastases are composed of either rhabdomyosarcoma or undifferentiated spindle cell sarcoma without cysts or a cartilaginous component. Follow up with brain imaging studies is mandatory for these patients (10,11).

Some patients with PPB have increased risk for ovarian Sertoli-Leydig tumors, renal cystic nephromas, nodular hyperplasia and carcinoma of the thyroid gland, as well as an assortment of other rare extra-pulmonary benign or malignant neoplastic conditions. These increased risks are in part associated with the tumor suppressor gene DICER1 (5). As an RNaseIII, DICER1 is involved in regulating post-transcriptional gene expression through the production of microRNAs. DICER1 also contributes to the generation of siRNAs that modulate gene expression.

Among patients tested for germline DICER1 mutations, 66.9%-80% were positive, indicating that individuals affected by conditions that are typical of DICER1 mutations should be offered DICER1 analysis (7,8). However, negative results for DICER1 mutation may be due to mosaicism (7).

Pathogenic germline mutations in DICER1 are associated with hereditary cancers with different manifestations. In addition to conferring increased cancer risk for PPB, particularly Sertoli-Leydig cell tumors, individuals with pathogenic germline DICER1 variants may also develop lung cysts, cystic nephroma, renal sarcoma and Wilms tumor, nodular hyperplasia of the thyroid, nasal chondromesenchymal hamartoma, ciliary body medulloepithelioma, genitourinary embryonal rhabdomyosarcoma, and brain tumors including pineoblastoma and pituitary blastoma (12).

Here we present the case of a 4-month-old patient with type II PPB, which is an unusual presentation given that type II and type III PPB typically occurs in older children. Genetic analysis performed for this child was negative for germline DICER1 detection (7-9).

2. AIM

Our aim was to present a case of a 4 month-old infant with type II PPB, who had a negative result for DICER1 mutation in next generation sequencing. To detail the clinical presentation of this patient, we present radiographic and ultrasound findings and results of histopathological analysis, as well as genetic and scintigraphic findings and chemotherapy treatment.

3. CASE PRESENTATION

A four month-old boy was admitted to the Pediatric Intensive Care unit at our hospital due to cough, dyspnea, shortness of breath and cyanosis. On admission, the child was afebrile and exhibited tachydyspnea. He had normal blood pressure and heart rate of 144 bpm, but the SpO2 was low (86%).

The child had recently experienced respiratory problems that were resolved with inhalation therapy. There was no family history of genetic diseases. After normal results were obtained for laboratory tests, a thoracic X-ray showed a massive shadowing of the left hemithorax. Based on this finding and results of other diagnostic approaches a diagnosis of type II PPB was made (ICD-11, 8973/3). Despite the predisposition for PPBs tumors among families, in this patient genetic no family-related disease were reported. Using DNA isolated from blood samples collected in the presence of ethylenediaminetetraacetic acid (EDTA), we performed next generation sequencing (NGS) using a Miseq instrument (Illumina). The sensitivity of this test is reported as 99.99%. We compiled a molecular genetic test report that included sequence analysis of all coding exons and exon-intron boundaries of the DICER1 gene and found no evidence of mutation. Although the results of genetic testing were normal, a normal test result alone cannot eliminate a diagnosis of PPB, so additional testing was carried out.

There were no breath sounds in the right pulmonary area on auscultation. A chest x-ray revealed massive shadowing of the right lung (Figure 1).

Ultrasonography of the lung detected a 7.15 x 5.79 cm mass attached to the pleura that contained cystic lesions (Figure 2).

Evaluation of contrast CT indicated the presence of a mass on the right bronchus (Figure 3). Surgery was planned.

The surgery involved a radical resection of a 7.0 x 5.0 cm mass that included cystic lesions. Histopathological analysis of the mass confirmed that it contained stromal and epithelial components that had a high risk of proliferation. Immunohistochemistry for tumor markers characteristic of type II PPB tumors was performed with staining for: 1) Cyclin D1; 2) Cytokeratin 7 and...
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20; 3) P63; 4) P53; 5) TTF1; 6) WT1--; 7) Ki67; and 8) Napsin A. Tumor biomarkers are substances that are detected in blood of patients with malignant tumors. The detection of biomarkers is useful in all stages of cancer care, including early detection, diagnostic, evaluation of chemotherapy efficiency and the prediction of metastasis dissemination.

Scintigraphy revealed a suspicious lesion in the right corner of the scapula (Figure 4), but no metastatic lesions were observed in the head. This finding was verified by MRI.

Histopathological examination confirmed the diagnosis of type II PPB. The patient was scheduled to start chemotherapy in a specialized pediatric oncology center for treatment with the IVADo chemotherapy protocol comprising: ifosfamide and vincristine 0.025 mg/kg/day delivered as a bolus; 1.25 mg/kg/day doxorubicin given by iv over 15 minutes on 2 days; and cyclophosphamide at 40 mg/kg/day. These dosages are reduced by 50% relative to typical regimens since the patient was an infant. Genetic analysis of germ line DICER1 mutations by NGS was negative.

A second CT scan performed 2 months after initiating chemotherapy treatment revealed a new, growing mass (Figure 5). Shortly after the CT scan, the patient’s condition deteriorated with leukopenia and respiratory distress followed by sepsis and cardiopulmonary arrest.

4. DISCUSSION

PPB is a dysontogenetic neoplasm of childhood that involves the lung and/or pleura. PPB is classified into three groups as cystic (type I), mixed (type II), or solid (type III). Type I occurs in infants (9 to 10 months), whereas type II and III is typically seen in older children (median diagnosis ages, 34 and 44 months).

PPB occurs with equal frequency among boys and girls. The occurrence of PPB in a 4-month-old infant is particularly rare. This child had experienced respiratory problems that resolved with inhalation therapy and...
there was no family or genetic history of disease. A thoracic X-ray taken after normal laboratory test results were obtained showed a massive shadowing of the left hemithorax. Together with findings from ultrasonography, scintigraphy and CT scanning, a diagnosis of type II PPB was made. NGS showed no mutation in DICER1, and there was no familial history of DICER1 mutation.

A previous study showed 435 cases, and a central review confirmed 350 cases to be PPB; 85 cases (20%) were another entity. Of the 350 confirmed cases, 33% were type I or type I regressed (type I r), 35% were type II, and 32% were type III or type II/III. The 5-year overall survival (OS) rate for type I/Ir patients was 91%; all deaths in this group were due to progression to type II or III disease. The 5-year OS rate for type II and type III disease was 71% and 53%, respectively. Disease-free survival (DFS) was also significantly better for type II versus type III (P = 0.0002) at 59% and 37%, respectively. PPB type was the strongest predictor of disease outcome. Of 97 patients tested, 66% had a heterozygous germline DICER1 mutation. However, in this patient subset the DICER1 germline mutation status was not related to disease outcome (14).

The gene encoding DICER1 maps to chromosome 14 q. DICER1 is a tumor suppressor gene that is inherited in an autosomal dominant manner. Mutations in this gene are associated with multiple types of tumors in the pediatric population, including PPB (15). Although >80% of patients with PPB carry a mutation in DICER1 (16), many individuals who carry DICER1 mutations never develop tumors due to a low penetrance of around 15% (17).

In practice, DICER1 gene mutation analysis should be offered to patients suspected of having DICER1 tumor-related syndromes, and if the child is found to have a germline mutation, testing should also be offered to the parents.

Taking into account the relationship between PPB and DICER1 gene mutation as well as the low penetrance, the finding that our patient was negative for a DICER1 mutation was perhaps not surprising. However, the rapid transformation from type I to type II is unusual for such a young patient and additional investigation is needed to assess whether a DICER1-independent mechanism was involved in this rapid tumor cell transformation, which can also be a good prognostic factor. As yet there is no specific molecular marker that can be used for immunohistochemical diagnosis of PPB (18), even though for the case we describe here some markers that are indicative of proliferation of differential tumor tissue were positive. Despite undergoing chemotherapy for two months, the condition of this patient worsened due to leukopenia and development of a new mass that eventually occupied the entire left hemithorax that led to respiratory insufficiency, sepsis and death. Although type II and type III PPB do have a poorer prognosis than type I, in this patient we observed no signs of metastasis to the brain, the most common site seen for PPB (18).

The early form of PPB, cystic type I, can be clinically and pathologically deceptive, due to its resemblance to some developmental lung cysts. A study reviewing 51 cases of type I PPB and 6 lung cysts from relatives of children with PPB showed rhabdomyoblasts and cartilage nodules in 49% and 40% of cases, respectively. In addition, varying numbers of delicate, multimolecular cysts comprising primitive mesenchymal cells beneath a benign epithelial surface were observed. In the youngest subset of patients, from birth to two months of age, tumors were more uniform in terms of composition and cellularity relative to those in older groups (19).

Although type II and type III PPB both have high death rates, type II disease has a lower rate of poor outcomes. PPB type and the presence of distant metastasis at diagnosis are the most important prognostic factors related to treatment outcomes. For type II and type III patients, surgery and chemotherapy are critical components that increase the likelihood of achieving a cure. Current attempts to screen DICER1 mutation carriers for cystic PPB at a young age may permit earlier detection of PPB type I, whereas subsequent surgical resection may prevent disease progression to types II and III that have higher morbidity and mortality (20, 21).

Around 90% of patients with type I PPB can be successfully treated, compared to 50%-70% of those with type II or type III disease. Type I PPB can recur as type II or type III PPB (22).

The 4 month-old patient described in this case study indicates that PPB can rapidly transform to type II disease in the absence of DICER1 mutation, familial history and low penetrance.

5. CONCLUSION

Pleuropulmonary blastoma is a rare but highly aggressive neoplasm in children. Young children with Type I or Type I r PPB may experience progression.

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