Case Report
Pediatric atypical neuroblastoma: three cases report and a literature review

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Abstract: Atypical neuroblastoma (NB) refers to the primary onset of NB without a detectable mass in common sites, but in bone marrow or/and other metastasis parts. For a better elucidation and defining atypical NB, our experiences in three unusual cases of Chinese atypical NB are reported on this paper. Clinically, the first case had no other parts of the tumor infiltration in addition to bone marrow, but the second and third case was presented with the metastasis sites of NB including both bone marrow and bone. Usually in China, treatments of radiotherapy, surgery and hematopoietic stem cell transplantation (HSCT) may not suitable for patients with atypical NB, likely due to the absent of visible solid mass and sometimes without the drug approval of the conditioning regimen like melphalan, so, aggressive chemotherapy seems to be the effective first-line choice. Our experiences show that atypical NB might be associated with a worse outcome than typical NB despite extremely aggressive treatments. Several important aspects of diagnosis and management of atypical NB are highlighted in the following three case reports.

Keywords: Atypical, neuroblastoma, child

Introduction
Neuroblastoma (NB) deriving from neural crest cells that destined for anywhere in the body associated with sympathetic nerve system [1, 2], is the most common extracranial tumor diagnosed as young children, and account for 8-10% of all childhood cancers [3]. A general consensus about management of NB includes multimodal combination therapy such as chemotherapy, radiotherapy, surgery and hematopoietic stem cell transplantation (HSCT). Most, some NB eventually develops progressive disease and confers overall poor prognosis, more than 50% of NB patients still die of this disease [4].

Atypical NB is a rare tumor followed by an atypical course and variable presentation, to the best of our knowledge, this is the first three cases report of atypical NB on a single center. Till now, there is no literature describing the significant differences including the clinical profiles, laboratory findings, biologic prognostic factors, treatment and outcome between NB and atypical NB, therefore the purpose of this study is to contribute to a better delineation of the heterogeneous characteristics of this rare entity and figures prominently in distinguishing between the diagnoses of NB and atypical NB.

Case report
Case 1
The first case, a 4-year-old male presented with a primary complaint about pain in left knee joint and pale over a 7-month period and low fever for two days (Table 1). A physical examination showed moderate anemia appearance, the bean-size cervical lymph nodes, mild hepatosplenomegaly. There was mild tenderness of left knee on palpation. The abdomen was soft and no additional mass was palpable. The rest of the physical examination was not special. Laboratory tested results of case 1 was...
## Table 1. Summary of the clinical files

<table>
<thead>
<tr>
<th></th>
<th>Case 1</th>
<th>Case 2</th>
<th>Case 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender</td>
<td>Male</td>
<td>Male</td>
<td>Male</td>
</tr>
<tr>
<td>Age (years)</td>
<td>4</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Metastasis</td>
<td>Bone marrow</td>
<td>Bone marrow and bone</td>
<td>Bone marrow and bone</td>
</tr>
<tr>
<td>Clinical</td>
<td>Pain in left knee joint and pale</td>
<td>Recurrent bilateral knee pain with fever</td>
<td>Pain in left hip joint, fever and pale</td>
</tr>
<tr>
<td>Neuroblastoma cell % in bone marrow</td>
<td>67</td>
<td>41</td>
<td>56.5</td>
</tr>
<tr>
<td>MYCN</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Disease status of primary at end of induction chemotherapy</td>
<td>Complete remission</td>
<td>Partial remission</td>
<td>Partial remission</td>
</tr>
</tbody>
</table>
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Furthermore, blood chemistry tests, which included aspartate aminotransferase (AST), alanine aminotransferase (ALT), lactate dehydrogenase (LDH), creatinine, alpha fetoprotein (AFP) and carcinoembryonic antigen (CEA), were all normal. Meanwhile, bone marrow smears (Figure 1A, 1B) presented that the ratio of characteristic NB cells was 67%, these cells typically forming Homer-Wright rosettes. Genetic testing using fluorescence in situ hybridization presented that absence of MYCN amplification in the metastatic tumor cells in bone marrow. Chest and abdomen computed tomography (CT) scan (Figure 2A) revealed splenomegaly and multiple small lymph nodes enlargement in the armpits and retroperitoneum, no infiltration of the lungs, mediastinum and bilateral adrenalectomy. Total body

### Table 2. The course of induction chemotherapy

<table>
<thead>
<tr>
<th>Course</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
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</thead>
<tbody>
<tr>
<td>Protocol</td>
<td>C</td>
<td>C</td>
<td>P</td>
<td>C</td>
<td>P</td>
<td>C</td>
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<td>V</td>
<td>P</td>
<td>V</td>
<td>P</td>
<td></td>
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</tr>
</tbody>
</table>

CAV: Cyclophosphamide + Pirarubicin + Vincristine, PVP: Cisplatin + Etoposide, CT: Cyclophosphamide + Topotecan.

as follows: white blood cell count, $5.42 \times 10^9/L$ (normal range, $5.11 \times 10^9/L$), decreased hemoglobin, $97 \text{ g/L}$ (normal range, $110-130 \text{ g/L}$), platelet count, $389 \times 10^9/L$ (normal range, $100-300 \times 10^9/L$), increased serum NSE, $274.2 \text{ ng/ml}$ (normal range $\leq 16.3 \text{ ng/ml}$), increased Urine VMA/Cr, $150$ (normal range $\leq 10$). Furthermore, blood chemistry tests, which included aspartate aminotransferase (AST), alanine aminotransferase (ALT), lactate dehydrogenase (LDH), creatinine, alpha fetoprotein (AFP) and carcinoembryonic antigen (CEA), were all normal. Meanwhile, bone marrow smears (Figure 1A, 1B) presented that the ratio of characteristic NB cells was 67%, these cells typically forming Homer-Wright rosettes. Genetic testing using fluorescence in situ hybridization presented that absence of MYCN amplification in the metastatic tumor cells in bone marrow. Chest and abdomen computed tomography (CT) scan (Figure 2A) revealed splenomegaly and multiple small lymph nodes enlargement in the armpits and retroperitoneum, no infiltration of the lungs, mediastinum and bilateral adrenalectomy. Total body
skeletal scintigraphy and abdominal ultrasound both showed no pathologic findings. X-ray film of the chest showed bronchopneumonia and no mediastinum mass.

Treatment was limited to nine courses of chemotherapy with high-risk NB induction protocol (SMHPO-N2011) described (Tables 2 and 3). Peripheral blood minimal residual disease (MRD) analyzed by flow cytometry detecting CD45-/CD56+/GD2+ tumor cells starting from the seventh course. If MRD > 1 × 10⁻⁴ it may be regarded as positive.

After two courses of chemotherapy, pain in left knee was in remission successfully, and superficial lymph nodes, the liver and the spleen were not palpable. From the fourth course, urine VMA (Table 4) continuously to be within normal limits, and the bone marrow smear (Figure 1C, 1D) revealed complete alleviation. From the sixth course, serum NSE (Table 4) drastically decreased to normal in the fourth course, while followed by a strong, continuous elevation from the seventh course. In the fifth course, PET/CT (Figure 4C) disclosed multiple residual tumor lesions that were not seen in the sixth course.

Nine courses of chemotherapy with protocol SMHPO-N2011 were performed. Peripheral blood MRD of NB was analyzed starting from the sixth course.

After one course of chemotherapy, ostealgia was complete in remission, from the fourth course, the bone marrow smear (Figure 3C, 3D) revealed complete alleviation, from the sixth course urine VMA (Table 4) got normal. Serum NSE (Table 4) drastically decreased to normal in the fourth course, while followed by a strong, continuous elevation from the seventh course. In the fifth course, PET/CT (Figure 4C) disclosed multiple residual tumor lesions that were not seen in the sixth course.

Case 2

The second case, a 3-year-old male was referred to our clinic for a workup, his chief complaint was, “recurrent bilateral knee pain with fever for one month.” On examination, the patient suffered from moderate anemia, and the neck examination was positive for bean-size lymph nodes. His abdomen was soft, no mass was palpable. Joints were no redness

and swelling, however, left knee joint tenderness was noted. His laboratory test results were as follows: white blood cell count of 5.04 × 10⁹/L, decreased hemoglobin of 90 g/L, decreased platelet count of 46 × 10⁹/L, increased serum NSE of 234.4 ng/ml, increased urine VMA/Cr of 28.5. The bone marrow aspirate (Figure 3A, 3B) revealed 41% typical NB cells with arranged in Homer-Wright rosettes. The MYCN amplification was negative. The chest and abdomen CT scans (Figure 4A) revealed augmented liver and multiple small lymph nodes enlargement in the armpits, no infiltration of the lungs, mediastinum and retroperitoneal. The infiltration in the left distal femur and the left proximal tibia, joint effusion in left knee were noted with magnetic resonance imaging (MRI) of the bilateral knee. PET/CT (Figure 4B) dated-September 2011 was obtained and it showed the diffusely increased metabolism of the bone marrow and bilateral femurs, enlargement of spleen and lymph nodes in bilateral neck, armpit, mesentery and pelvic cavity, compatible with tumor infiltration.

After one course of chemotherapy, ostealgia was complete in remission, from the fourth course, the bone marrow smear (Figure 3C, 3D) revealed complete alleviation, from the sixth course urine VMA (Table 4) got normal. Serum NSE (Table 4) drastically decreased to normal in the fourth course, while followed by a strong, continuous elevation from the seventh course. In the fifth course, PET/CT (Figure 4C) disclosed multiple residual tumor lesions that were not seen in the sixth course.

Table 3. The composition and usage of protocol

<table>
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<tr>
<th>Protocol name</th>
<th>Dosage and usage</th>
<th>Days</th>
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<tr>
<td>CAV Cyclophosphamide (CTX)</td>
<td>1.2 g/m².d, iv drip</td>
<td>d1-d2</td>
</tr>
<tr>
<td>Pirarubicin (THP)</td>
<td>25 mg/m².d, iv drip</td>
<td>d1-d3</td>
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<tr>
<td>Vincristine (VCR)</td>
<td>0.022 mg/kg.d (or 0.67 mg/m².d), iv drip</td>
<td>d1-d3</td>
</tr>
<tr>
<td>PVP Cisplatinum (DDP)</td>
<td>50 mg/m².d, iv drip</td>
<td>d1-d4</td>
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<tr>
<td>Etoposide (VP16)</td>
<td>200 mg/m².d, iv drip</td>
<td>d1-d3</td>
</tr>
<tr>
<td>CT CTX</td>
<td>1.2 g/m².d, iv drip</td>
<td>d1-d2</td>
</tr>
<tr>
<td>Topotecan</td>
<td>2 mg/m².d, iv drip</td>
<td>d1-d3</td>
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Table 4. The values of serum NSE, urine VMA and MRD in peripheral blood

<table>
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<tr>
<th>Course</th>
<th>NSE (ng/ml)</th>
<th>VMA/Cr</th>
<th>VMA (mg/24 H)</th>
<th>MRD ($\times 10^{-4}$)</th>
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<tbody>
<tr>
<td></td>
<td>Case 1</td>
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</tr>
<tr>
<td>1</td>
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<td>724.4</td>
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<td>2</td>
<td>32.5</td>
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<tr>
<td>9</td>
<td>75.4</td>
<td>20.7</td>
<td>16.9</td>
<td>-</td>
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</table>

Note: NSE (normal range ≤ 16.3 ng/ml); VMA/Cr (normal range ≤ 10); VMA (normal range: 5~15 mg); MRD (normal range < 1 $\times 10^{-4}$).

Figure 3. Bone marrow morphology in case 2. A: Before chemotherapy begins the ratio of characteristic NB cells was 41% (wright’s stain × 100). B: Before chemotherapy begins, the neoplastic cells with cell bodies varied in size, scant cytoplasm stained with blue and enlarged nuclei, typically formed Homer-Wright rosettes (wright’s stain × 1000). C and D: From the fourth course, the bone marrow smear revealed complete alleviation.

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at characterized by multiple osteolytic bone destruction and increased metabolism of the bilateral humerus, the sternum, the spine vertebral and accessories, the pelvis and the femurs. The last PET/CT scan (Figure 4D), performed after the entire induction chemotherapy, also showed bone invasion without remission in accord with the former (Figure 4C). In the sixth course, NB peripheral blood negative MRD value (Table 4) was $0.73 \times 10^{-4}$, unfortunately MRD became positive ($8.36 \times 10^{-4}$) in the ninth course. Eventually, the second case achieved partial remission, and then he abandoned subsequent treatment also for economic reason.

Case 3

The third case, a 3-year-old male with pain in left hip joint for two months history, repeated fever and pale for one month was referred to our hospital for a workup (Table 1). On examination, moderate anemia was observed. On palpation, there was no superficial lymph adenopathy, the abdomen was flat and no mass was palpable. The rest of the physical examination revealed no abnormalities. Laboratory findings of case 3 showed that white blood cell count was $6.97 \times 10^9$/L, decreased hemoglobin of 79 g/L, platelet count of $244 \times 10^9$/L, increased serum NSE of 724.4 ng/ml, increased urine VMA/Cr of 148. Simultaneously, bone marrow smears (Figure 5A, 5B) showed the ratio of characteristic NB cells was 56.5%. The MYCN amplification was negative. MRI of lumbar and pelvis revealed the significant infiltration in lumbar vertebral body, sacrum, bilateral iliac, ischium, pubis and bilateral femoral head. Abdominal ultrasound found multiple gallstones without other solid mass. PET/CT (Figure 6A) showed the diffusely increased metabolism of multiple bones with osteolytic lesion, increased metabolism of spleen and lymph nodes in left pelvic wall, all consistent with bone marrow involvement.

The nine courses of intensive chemotherapy with protocol SMHPO-N2011 were performed, after one course of chemotherapy, ostealgia was complete in remission, from the fifth
course, the bone marrow smear (Figure 5C, 5D) revealed complete remission. In the fourth course, serum NSE (Table 4) dropped to normality, but rapidly rising from the fifth course, in the second course, urine VMA (Table 4) got normal, but from the third course, urine VMA turned to be positive. In the third and the last course, PET/CT (Figure 6B, 6C) showed a complete response in the multiple bone invasion lesions and lymph nodes, enlarged spleen but with normal metabolism. Finally, the third case achieved partial remission after the entire induction chemotherapy, and he experiences consolidation chemotherapy at present.

Discussion

Previously reported cases of atypical NB were rare. On the basis of our retrospective literature review showing that there are only 12 case reports about the atypical NB [5-9]. Darren Salmi and colleagues described a 20-month-old female with unknown primary site detect-
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Figure 5. Bone marrow morphology in case 3. A: Before chemotherapy begins the ratio of characteristic NB cells was 56.5% (wright’s stain × 100). B: Before chemotherapy begins, the neoplastic cells with cell bodies varied in size, scant cytoplasm stained with blue and enlarged nuclei, typically formed Homer-Wright rosettes (wright’s stain × 1000). C and D: From the fifth course, the bone marrow smear revealed complete alleviation.

Figure 6. Image results in case 3. (A) PET/CT in case 3 initially showed the diffusely increased metabolism of multiple bones with osteolytic lesion, increased metabolism of spleen and lymph nodes in left pelvic wall, all consistent with bone marrow involvement. (B) PET/CT of case 3 in the third course showed a complete response in the multiple bone invasion lesions and lymph nodes, enlarged spleen but with normal metabolism. (C) PET/CT of case 3 in the last course was similar to the former (B), with bone involvement complete remission.

ed based on imaging studies included CT and $^{123}$ I-MIBG scan [6]. Similarly, Ki Woong Sung reported that in 143 NB two patients originated from unknown primary sites in 1.4% of cases [8]. In these cases it remains unclear where neoplasms originated after patients had been diagnosed as NB. Consequently, the primary occult tumor may not large enough for detection based on imaging studies. Furthermore, literature review has revealed that the standard diagnostic criterion and clear definition are still enigmatic. According to the special characteristics of these cases of pediatric NB described here, we try to define the atypical NB as the primary onset of NB without a detectable mass in common sites based on imaging studies, but in bone marrow or/and other metastasis parts.
Bone and bone marrow metastases from unknown primary tumor commonly appear as pain in joint or pale. Unlike atypical NB, NB usually has definite primary sites. Hence, the diagnosis of atypical NB depends on exclusion of definite primary sites NB. The diagnosis is facilitated by significantly clinical features such as no detectable primary tumor via conventional imaging methods at diagnosis, but typical NB cells were found in the metastasis or infiltration areas, and the specific NB tumor markers (such as NSE and urine VMA, etc) were positive [10]. However, equivocal features may be encountered, and the main differential diagnosis encompasses hematological disease, rheumatic diseases and infectious diseases. Among them, pale may be readily misdiagnosed as leukemia while overlooking atypical or discordant clinical features. It is important to identify those patients from hematological disease and morphology of medullary cells may be the effective tool for this purpose. Strikingly, emphasis of the atypical NB diagnosis should be placed on no substantial mass in common sites as the classical primary NB as the key and it should avoid becoming a major obstacle to make a definitive diagnosis.

Serum NSE and urine VMA are relatively valuable and specific tumor markers of NB [11, 12]. The serum NSE > 200 ng/ml is strongly associated with a worse outcome only in patients with stage IV and MYCN amplification negative NB, and that serum NSE is a limited prognostic marker in the typical NB risk stratification [13]. In our study, all the atypical NB patients showed the serum NSE increased significantly during the first visit and the trend of it was positively correlated with the disease severity and activity. Generally, the urine VMA as catecholamine metabolites is often helpful and has been proved as a valuable marker for predicting clinical behavior of NB [14]. There is no difference in the clinical value between 24 hours for testing urine VMA and single urine testing urine VMA/Cr [15]. Although urine VMA is widely used and valuable in the diagnosis of NB, the trend of urine VMA or urine VMA/Cr were not in accordance with the undesired prognosis in our patients. Compare with urine VMA or urine VMA/Cr, serum NSE was comparatively favorable prognostic indicator in atypical NB although it was less specific in general NB. A large-scale study will be necessary to determine if urine VMA is in a limited sense in atypical NB.

Peripheral blood MRD of NB figures prominently in finding occult tumor, significantly improving the sensitivity and accuracy, and further suggesting tumor recurrence or metastasis [16, 17], and has been reported as a significant prognostic factor for patients with NB [18]. Another provocative finding is that that MRD was constantly monitored for four months and widely available to evaluate prognosis [19, 20]. Virtually all NB tumor cells express CD56 and GD2 [21, 22], though phenotype of CD56+/CD45- is not unique to NB, the GD2 is highly expressed on NB cells and don’t express on cells of human bone marrow and peripheral blood make it as a specific marker for NB MRD detection. In our study, peripheral blood MRD analyzed by flow cytometry detecting CD45-CD56+/GD2+ tumor cells as previous report [23, 24], especially, the various trend of MRD values post-chemotherapy of case 1 and 2 consistent with worse prognosis of case 2. So, peripheral blood MRD could be considered a useful marker for evaluating prognosis and monitoring treatment response of atypical NB.

Amplification of the MYCN occurs in 20% of NB, is the most unfavorable prognostic factors and highly correlated with tumor aggressiveness [25], in this report, all three cases the amplification of the MYCN was negative, it seems that the MYCN status is quite limited correlating with clinical behavior of atypical NB.

Atypical NB is a rare subtype of NB. Although our study was limited to only three cases, they showed that PET/CT as a non-invasive technique can provide better visualization of the anatomic extent and will most likely become an option for monitoring treating response of atypical NB. Treatment of atypical NB in China particularly presents a great challenge, due to lack of solid mass and transplant related drugs like melphalan, while conventional intensive chemotherapy seems the only way for controlling the atypical NB. Although intensive chemotherapy was used, the majority of our patients showed a temporary and poor response. Since there is no subsequent consolidation therapy (for financial reasons) the case 1 relapsed, and the other two patients (case 2 and case 3) merely achieved partial remission after induction chemotherapy.
Conclusion

In our limited experience, we found that despite intensive chemotherapy the patient with atypical NB usually represented a worse clinical course and poor prognosis especially in those with bone metastases. This manuscript emphasizes that the importance of distinguishing atypical NB from typical NB lies in the fact that the atypical NB may have a much worse clinical behavior, on the other hand, the combined application of serum NSE, urine VMA, MRD and PET/CT are best regarded as an assistive technique for the diagnosis and monitoring of the atypical NB. In addition, as the data are limited, a large-scale study is required to investigate whether MYCN status and urine VMA are quite limited in correlating with clinical behavior of atypical NB.

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Disclosure of conflict of interest

None.

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References


