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ORIGINAL ARTICLE

Ultrasound-guided core needle biopsy in the diagnosis of neuroblastic tumors in children: a retrospective study on 83 cases

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Abstract
Aim Ultrasound-guided biopsy technique with the large-core needle has widely been applied in the diagnosis of adult abdominopelvic cavity, thyroid, and neck tumors. There are few reports on ultrasound-guided biopsy using large-core needle in pediatric abdominopelvic cavity tumors. This study was to evaluate the ultrasound features and the diagnostic value of ultrasound-guided core needle biopsy for pediatric neuroblastic tumors.
Methods The pediatric patients with neuroblastic tumor that underwent ultrasound examination and ultrasound-guided core needle biopsy from January 2009 to November 2015 were reviewed. A minimum of two cores in each case was obtained. The biopsy results were confirmed by subsequent surgical histopathology. The ultrasound features and the diagnostic accuracy of ultrasound-guided core needle biopsy were evaluated.
Results Eighty-three patients were enrolled into the study. Conventional ultrasound examination showed irregular hypoechoic or mixed echo masses and calcification and liquefied necrosis. The diagnostic accuracy of ultrasound-guided core needle biopsy was 96.4% (80/83). Three cases were misdiagnosed because of inadequate tissue sample. No serious complication, infection, or needle track seeding occurred.
Conclusions Ultrasound-guided core needle biopsy seems to be an accurate, minimally invasive, and safe diagnostic method of pediatric neuroblastic tumor.

Keywords Abdominopelvic tumor · Core needle biopsy · Diagnostic accuracy · Neuroblastoma

Abbreviations

GN	Ganglioneuroma
GNB	Ganglioneuroblastoma
NB	Neuroblastoma

Introduction

Neuroblastic tumor is a common extracranial solid tumor in children and includes three categories: ganglioneuroma (GN), ganglioneuroblastoma (GNB), and neuroblastoma (NB) [3, 15]. The pathological characteristics and biological behavior of neuroblastic tumors vary greatly due to different degrees of differentiation. GN consists of mature ganglion cells and is benign tumor. NB contains more than 50% immature neuroblasts and is the least differentiated and the most malignant tumor among the above three types [7, 12]. GNB consists of mature ganglion cells and immature neuroblasts. NB accounts for 8–10% children malignant tumor and ranks the third position following the tumors of blood system and the tumors of central nervous system [10, 15]. As a neuroendocrine tumor, it frequently originates in adrenal glands, and can also develop in the nerve tissues of the neck, chest, abdomen, and pelvis.

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NB has varied clinical manifestations and the tumor location is often hidden. It is critically important to make precise pathological diagnosis before treatment. Imaging methods, including CT, MRI, and ultrasound, are often used to provide the information of the tumor location, size, internal structure, and blood supply. Among them, ultrasound has the advantage of low cost and no radiation damage [4]. However, it is difficult to achieve a diagnosis using imaging methods alone. The pathological biopsy is necessary and the imaging-guided needle biopsy can also avoid the open surgery. Ultrasound is regarded as the optimized method to guide biopsy which provides a multi-perspective, multi-dimensional view, and real-time monitoring of the tumor location and the course of the biopsy needle tip [1]. It is also available even in the small tumors (<1 cm). Ultrasound-guided biopsy technique has widely been applied clinically, using the large-core needle or fine needle. Compared with fine needle, the large-core needle has many advantages, including rapid sampling with automatic biopsy gun and obtaining adequate tissue [6]. It is reported that the large-core needle has been used in diagnosing adult abdominopelvic cavity, mediastinum, breast, thyroid, and neck tumors [4, 16, 17].

Tissue diagnosis is mandatory prior to the treatment of abdominal or pelvic lesions in children. As far as we know, there are few reports on ultrasound-guided biopsy using large-core needle in pediatric abdominopelvic cavity tumors. In this study, 83 cases of pediatric abdominopelvic cavity neuroblastic tumor were retrospectively reviewed. The main endpoints were the ultrasound features and the diagnostic accuracy of ultrasound-guided core needle biopsy, and the complications following the biopsy.

Methods

Patients

This study was approved by the ethic committee of the hospital and the signed informed consent was obtained from each patient. The pediatric patients with surgically removed neuroblastic tumors were confirmed pathologically from January 2009 to December 2015; their clinical data and ultrasound-guided biopsy were collected and evaluated retrospectively. The clinical symptoms were abdominal pain, abdominal distension, abdominal mass, fever, anaemia, angular, weakness, pain in the lower limb, etc. The inclusion criteria were abdominal neuroblastic tumor ≥3.0 cm, histopathologically confirmed tumor after the surgery, and successful core needle biopsy. The exclusion criteria were blood platelet counts <50,000/ml and/or international normalized ratio (INR) >1.6. All patients underwent preoperative ultrasound examination and ultrasound-guided core needle biopsy.

Ultrasound examination

The patients were not allowed to take food within 6 h before ultrasound examination. The patients were under supine or lateral position. Ultrasound Diagnostic System (iU22, C5-1 Probe, 3–5 MHz, Philips, Holland) and Color Doppler Ultrasound Diagnostic System (Logic 9, convex array probe, 3.5–5.0 MHz, GE, USA) were used. The image depth was adjusted depending on the patient's figure and the neoplasm size to obtain the best images with clear tumor and pericarcinous tissue. Continuous scan of each aspect was performed to assess the tumor location, size, shape, boundary, internal echo, calcification, and its relationship with the blood vessels of the surrounding organs. Tumor blood flow was classified according to the Adler method [2]: Degree 0 shows no obvious tumor blood flow signal; Degree I shows a small amount of blood flow with dot signal; Degree II displays moderate amount of blood flow with one main blood vessel (its length was above the radius of the tumor) in each cross section or with 2–4 dot blood flow signals; Degree III is with rich blood flow and more than three blood vessels or diffused blood vessels. The categories of ultrasound echo were defined as homogenous (the echo was homogenous), partially inhomogenous (there were some slight patchy inhomogenous echo), and dispersely inhomogenous (there were many inhomogenous echo areas continually connected). The calcification degree was defined as scattered dot calcification (there were a few sporadically distributed dot-shape calcification), diffused dot calcification (there were many dot-shape calcification), and patchy calcification (there were some patchy calcification).

Ultrasound-guided biopsy

The biopsy was performed by two sonographers with about 7 years' experience. The puncture point and the approach were determined first by the probe (LOGIQ 9, 3.5C, GE, USA; iU22, C5-1, Philips, Holland), avoiding the great vessels and the surrounding organs. After skin sterilization, local anaesthesia with 10% chloral hydrate calm and 2% lidocaine, local anaesthesia with ketamine (2 mg/kg/h) and lidocaine, or general anaesthesia with atropine (0.01–0.02 mg/kg), ketamine (2 mg/kg/h), and propofol (6–8 mg/kg/h) was performed. The patients under 2 years were performed with general anaesthesia. And then, the patients underwent biopsy. The sonographer operated the ultrasonic instrument panel by one hand and used the sterile probes to besmear coupling agent by the other

hand. The patient kept breathless, while the other sonographer held the automatic biopsy gun (MG15–22, Bard, USA) and rapidly ejected the disposable biopsy needle (18G, Bard MAGNUM, USA) into the mass and quickly pulled it out. Disinfection was immediately done using entoiodine and the wound was pressed to prevent bleeding and bandaged with adhesive plaster and elastic bandage. At least two cores of tissue (15–22 mm long) were obtained from each patient. The tissue was fixed with 10% formaldehyde for further histopathological examination. After the biopsy, the patients were under close observation for half an hour.

Histopathological examination

The obtained samples were examined in the department of pathology in our institute. A minimal amount of tissue (2–3 mm of the core) was set aside and preserved for future diagnostic studies. A minimal amount of tissue was submitted into RPMI-1640 cell medium for a combination of cytogenetic diagnosis, if necessary. The remaining tissue was fixed in 10% buffered formalin prior to paraffin embedding. The tissue sections (3 μm thick) were prepared from paraffin-embedded tissue blocks and mounted on glass slides. The mounted tissue sections were stained with hematoxylin and eosin after dewaxing with xylene and gradient ethyl alcohol and washing by distilled water. The histopathological examination was conducted by pathologists.

Statistical analysis

All measured quantitative data were presented as mean ± SD. The numeration data were expressed as number of cases and analyzed by the Chi-square test. $P < 0.05$ was considered as statistical significance. The statistical analysis was performed with SPSS 17.0 (SPSS Inc, Chicago, IL).

Results

Patients' characteristics

Eighty-three patients (49 males, 34 females) met the inclusion criteria and were enrolled into the study. There were 16 patients under 1 year, 17 patients 1–2 years old, 30 cases 3–5 years old, 14 cases 6–9 years old, and 6 cases 10–13 years old. Patients' baseline characteristics are presented in Table 1. There are 6 cases of GN, 20 cases of GNB, and 57 cases of NB according to the postoperative pathological analysis. As for the anaesthesia method, 45 cases underwent local anaesthesia and 38 cases underwent general anaesthesia.

Table 1 Baseline characteristics and clinical symptoms of the patients

Characteristics	
Male/female	49/34
Age	0.8–13 years old
GN/GNB/NB	6/20/57 cases
Abdominal pain, distension and mass	44 cases
Fever	17 cases
Anaemia and feebleness	10 cases
Lower limb pain	7 cases

GN ganglioneuroma, *GNB* ganglioneuroblastoma, *NB* neuroblastoma

Features of the ultrasound examination

The ultrasound examination (Table 2) showed that the primary sites were located at adrenal gland (29 cases, including 14 cases in left side, 11 cases in right side, and 2 cases in bilateral sides), in retroperitoneum (48 cases) and abdominopelvic cavity (6 cases). The two-dimensional ultrasound and color Doppler showed that the average tumor size was $9.7 \times 7.3 \times 5.7$ cm^3 (the smallest one was $3.3 \times 2.7 \times 2.6$ cm^3 and the largest one was $18.4 \times 15.0 \times 9.0$ cm^3).

The ultrasound examination showed irregular hypoechoic or mixed echo masses. Punctiform or patchy calcification was observed in 64 cases, and liquidity area was observed in 34 cases. The tumors growing across the midline were observed in 32 cases and the masses grow encasing the large abdominal blood vessels were observed in 18 cases. There were 17 cases whose liver or kidney was squeezed, among whom seven cases appeared hydronephrosis due to the tumor infringement. Retroperitoneal lymph node enlargement occurred in 20 cases and liver metastasis occurred in four cases. As for tumor blood flow, there were 13 cases of Degree 0, 24 cases of Degree II, and 46 cases of Degree III according to the Adler method.

In GN-GNB-NB ultrasonographic images, the general trend of sonographic changing can be observed from homogenous to partially inhomogenous, dispersely inhomogenous, and dispersely inhomogenous wth liquid area (ranksum test, $P = 0.001$). With regard to the calcification, a similar trend can be observed from scattered dot calcification to diffused dot calcification and patchy calcification (ranksum test, $P = 0.003$). In the six cases with GN (Figs. 1a, b; 2a, b; 3a, b), there were three cases (3/6, 50.0%) with homogenous echo and three cases (3/6, 50.0%) with partially inhomogenous echo. In the 20 patients with GNB, there were three cases (3/20, 15.0%) with homogenous echo, four cases (4/20, 20.0%) with partially inhomogenous echo, six cases (6/20, 30.0%) with dispersely inhomogenous echo, and seven cases (7/20,

Table 2 Features of the ultrasound examination

	Single/multiple	Site (adrenal/retroperitoneal/pelvic cavity)	Border (clear/unclear)	Shape (regular/irregular)	Echo (homogenous/partially inhomogenous/dispersely inhomogenous)
GN, 6 cases	4/2	3/3/0	6/0	5/1	3/3/0
GNB, 20 cases	8/12	7/11/2	7/13	8/12	3/4/13
NB, 57 cases	25/32	19/34/4	16/41	12/45	0/9/48

GN ganglioneuroma, *GNB* ganglioneuroblastoma, *NB* neuroblastoma

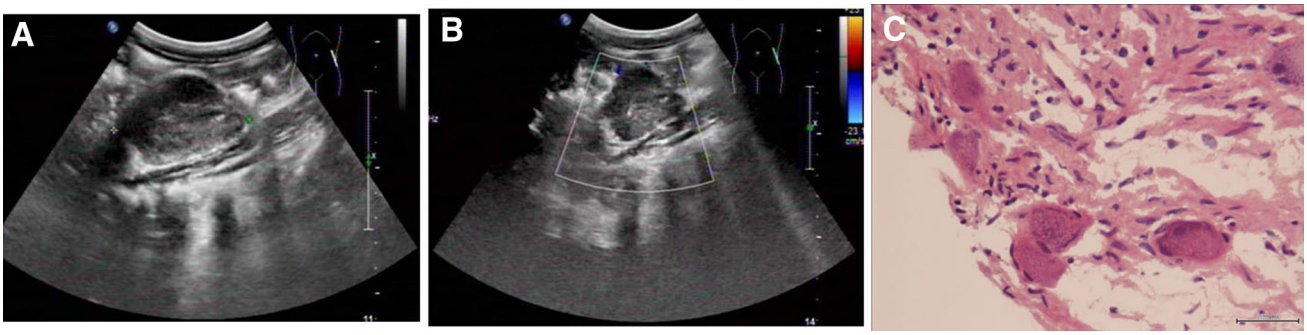

Fig. 1 Ultrasound examination and biopsy of a patient (female, 6 years old) with ganglioneuroma. **a**, **b** It showed the solid retroperitoneal tumor in the left, with inhomogenous hypoecho, unclear border, and punctiform calcification. **c** Pathological analysis of the biopsy suggested that it was a retroperitoneal ganglioneuroma (amplification ×400)

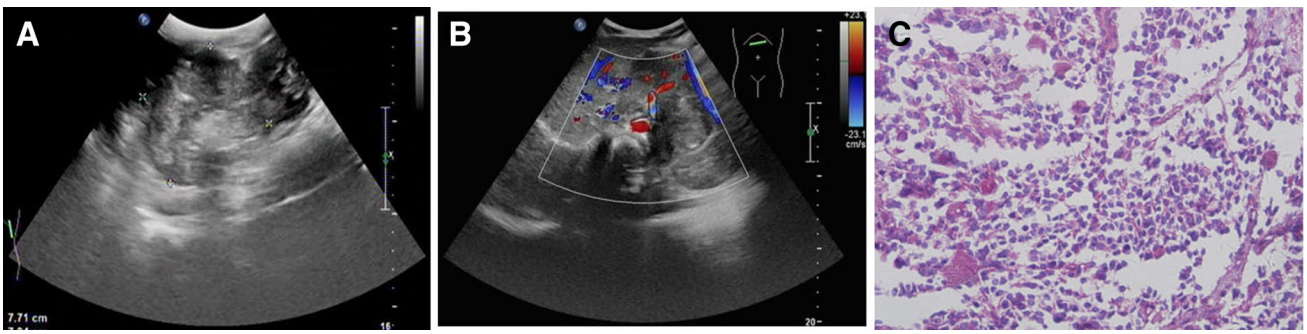

Fig. 2 Ultrasound examination and biopsy of a patient (male, 3 years old) with ganglioneuroblastoma. **a**, **b** It showed that the solid tumor located between the spleen and kidney and in the left upper abdomen, and the tumor was with inhomogenous echo, unclear border, patchy calcification, and signals of thick blood flow. **c** Biopsy suggested that it was a left retroperitoneal ganglioneuroblastoma (amplification ×400)

35.0%) dispersely inhomogenous echo with liquid area. In the 57 patients with NB, there were nine cases (9/57, 15.8%) with partially inhomogenous echo, 18 cases (18/57, 31.6%) with dispersely inhomogenous echo, and 30 cases (30/57, 52.6%) dispersely inhomogenous echo with liquid area. In the six cases with GN, there were two cases (2/6, 33.3%) with scattered dot calcification and one case (1/6, 16.7%) with diffused dot calcification. In the 20 patients with GNB, there were five cases (5/20, 25.0%) with scattered dot calcification, four case (4/20, 20.0%) with diffused dot calcification, and five cases (5/20, 25.0%) with patchy calcification. In the 57 patients with NB, there were 21 cases (8/57, 14.0%) with scattered dot calcification, 13 case (13/57, 22.8%) with diffused dot calcification, and 26 cases (26/57, 45.6%) with patchy calcification (Table 2).

Sixty-eight cases were diagnosed as neuroblastic tumor with diagnostic accuracy of 81.9% (68/83) according to the results of ultrasound examination (Table 3). Thirteen cases were misdiagnosed as nephroblastoma (3 cases), hepatoblastoma (1 case), metastatic tumor (4 cases), lymphoma (3 cases), and neurogenic tumor (2 cases), and two cases were undiagnosed.

Diagnostic accuracy of ultrasound-guided biopsy

Three cores of tissue were obtained on average (range 2–4 cores). Adequate tissue was obtained for pathological

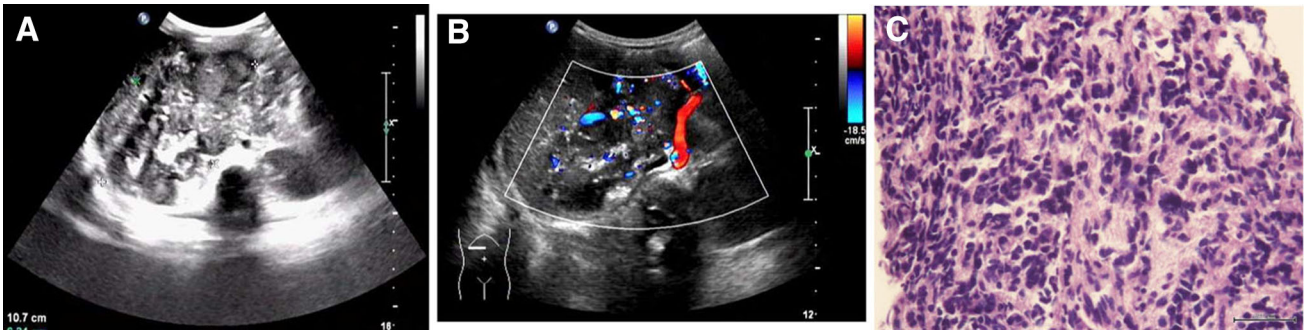

Fig. 3 Ultrasound examination and biopsy of a patient (female, 3 years old) with neuroblastoma. **a**, **b** It showed the solid tumor in the retroperitoneal, with inhomogenous echo, calcification, and rich blood flow signal in the tumor. **c** Biopsy suggested that it was a right retroperitoneal neuroblastoma (amplification ×400)

Table 3 Diagnostic result of postoperative pathology and ultrasound examination

Postoperative pathology	Cases number	Ultrasound examination	Cases number
NB	57	Neuroblastic tumor	47
		Nephroblastoma	2
		Hepatoblastoma	1
		Metastatic tumor	3
		Lymphoma	1
		Other undetermined neurogenic tumor	1
		Undetermined	2
GNB	20	Neuroblastic tumor	17
		Lymphoma	2
		Nephroblastoma	1
GN	6	Neuroblastic tumor	4
		Metastatic tumor	1
		Other undetermined neurogenic tumor	1

GN ganglioneuroma, *GNB* ganglioneuroblastoma, *NB* neuroblastoma

Table 4 Diagnostic result of ultrasound-guided biopsy and ultrasound examination

Postoperative pathology	Cases number	Ultrasound examination	Cases number
NB	57	NB	54
		GN	1
		Neuroendocrine small cell malignant tumor	1
		Small-round cell malignant tumor	1
GNB	20	GNB	20
GN	6	GN	6

GN ganglioneuroma, *GNB* ganglioneuroblastoma, *NB* neuroblastoma

analysis (Figs. 1, 2, 3c) in 80 cases (80/83, 96.4%), and the rest three cases with little tissue obtained were misdiagnosed as ganglioneuroma, small-round cell malignant tumor, and neuroendocrine small cell malignant tumor. The diagnosis accuracy was 96.4% (80/83; Table 4). No patients underwent repeat biopsies in the same lesion. In this study, 21 cases were treated with chemotherapy after the biopsy and before the surgery. Among the 21 cases, the biopsy of one patient before chemotherapy indicated ganglioneuroblastoma, after the chemotherapy and surgery, the pathological examination indicated ganglioneuroma.

Three patients showed mild complication (3/83, 3.6%) resulted from biopsy, including two cases of post-biopsy pain in the puncture site and one case of bleeding (about 15 ml) in the puncture site. All of them made a prompt recovery. No infection or needle track seeding occurred. No patient needed any peri-procedural transfusion.

Discussion

Tissue diagnosis is critically important before the treatment of pediatric neuroblastic tumor. This study showed that the ultrasound-guided needle biopsy provided a rather accurate diagnosis of neuroblastic tumor.

This conventional ultrasound examination is the first choice for the diagnosis of the abdominal pelvic neuroblastic tumor, such as the retroperitoneal, pelvic, and adrenal gland neoplasm. Due to the insidious onset and the depth of the lesion site, neuroblastoma is usually characterized by large volume and irregular shape [9]. There were 23 cases with huge tumors (maximum diameter $\geq$10 cm) in this study. The huge tumors often squeeze the adjacent organs, grow across the midline, and circumvolute the large retroperitoneal blood vessels.

As for the diagnostic accuracy of ultrasound examination, it is 81.9% (68/83) with 13 cases misdiagnosed as nephroblastoma (3 cases), hepatoblastoma (1 case), metastatic tumor (4 cases), lymphoma (3 cases), and neurogenic tumor (2 cases), and two cases failed to make a diagnosis. The misdiagnosis might happen when there was the large tumor invading liver and kidney [8]. Therefore, it is hard to judge the pelvic tumor which usually has no special ultrasound imaging characteristics.

The diagnostic accuracy of the ultrasound-guided needle biopsy was 96.4% (80/83), which was in accordance with the previous report of 75–100% [13, 14]. There was one case of NB that was misdiagnosed as GN and two cases of NB that were misdiagnosed as small-round cell tumor and neuroendocrine small cell tumor. The three cases of misdiagnoses might by caused by the inadequate tissue sample. As the malignant degree of the neuroblastic tumor is closely related to the proportion of the various differentiated cells, sampling from different sites is necessary for accurate diagnosis. Besides, it is critical to sample from the inhomogenous echo area surrounding the tumor to obtain the malignant tissue. Hugossn et al. [6] reported that some small-round cell tumor in children was similar under electronic telescope; adequate sampling and immunohistochemical examination were necessary to get a precise diagnosis. And this was in accordance with this study. The misdiagnosis was attributed to insufficient viable tumor tissue being collected and the liquefied necrosis which was unfavorable for sampling. The accurate diagnosis could be probably achieved with more different sampling sites.

Mullassery et al. compared the accuracy between laparotomy biopsy and ultrasound-guided biopsy, and found that the two methods were comparable with the accuracy of 95% (20/21) and 94% (17/18), respectively [11]. Laparotomy biopsy can guarantee sufficient sampling for histological and molecular examination. However, laparotomy biopsy is usually accompanied by much trauma and complications, such as small intestinal injury and intestinal obstruction [5, 11]. Therefore, ultrasound-guided biopsy shows preferable advantages. It is important to keep in mind of the heterogeneity of neuroblastoma where the stromal cells, nerve cells, and differentiated cells are unevenly distributed. Thus, the biopsy should be performed in several different parts of the neuroblastoma [11]. Three cores of tissue were obtained from each patient on average (range 2–4). Another important aspect of the biopsy is sufficient tissue sampling to perform all the required biologic tests. Adequate tissue was obtained for the required biologic tests in 80 cases (80/83, 96.4%), and the rest three cases did not obtain adequate tissue and were misdiagnosed as ganglioneuroma, small-round cell malignant tumor, and neuroendocrine small cell malignant tumor. Among the three cases without enough sampling, two cases were with loose neoplasm structure and it was difficult to avoid the tiny liquid zone and get an unbroken tissue when performing the biopsy. The other case showed that the mass surrounded the retroperitoneal vessels and was squeezed by necrotic tissue; only little sample was obtained to avoid the spleen and kidney. According to our clinical experience, we suggested that the biopsy should be performed sampling from different areas, especially in the part with rich blood flow and the area with heterogeneity echo. The liquefied necrosis and patchy calcification should be carefully avoided.

There were three incorrect diagnoses in this study, suggesting that the ultrasound-guided core needle biopsy cannot be used as a gold standard. There were some limitations in this study. Only neuroblastic tumors were included in the present research. It is probably not very appropriate to consider the pathology report inaccurate when comparing biopsy at presentation to surgical resection specimen if chemotherapy has been given in between as the chemotherapy can change the degree of tumor differentiation. The biopsy suggested that it was ganglioneuroblastoma, and, after chemotherapy for four courses, the surgically removed tumor was proved to be ganglioneuroma according to the pathological analysis. Besides, the sample size was small and this is a retrospective study. It could be concluded that the ultrasound-guided core needle biopsy seems to be an efficient, minimally invasive, and safe diagnostic means of neuroblastic tumor in pediatric patients.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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