Title

Importance of Magnetic Resonance Imaging with Diffusion-weighted Imaging in Guiding Biopsy of Nodular Ganglioneuroblastoma: A Case Report

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Abstract

Background: Nodular ganglioneuroblastoma is a rare peripheral neuroblastic tumor of variable prognosis. Accurate diagnosis, staging and risk categorisation can be particularly challenging in patients with nodular ganglioneuroblastoma due to the inherent heterogeneity of these lesions.

Case presentation: We illustrate the use of diffusion weighted magnetic resonance imaging (DWI-MRI) to identify tumor nodules and guide tumor biopsy in a five-year old boy with a large abdominal tumor.

Conclusions: DWI-MRI was successful in detecting and guiding biopsy of a poorly differentiated neuroblastoma nodule within the context of a well differentiated ganglioneuroma, allowing the diagnosis and characterization of a ganglioneuroblastoma nodular, thus influencing the child's prognosis and treatment.

Key words: diffusion weighted imaging, magnetic resonance imaging, ganglioneuroblastoma nodular, neuroblastoma, ganglioneuroma, neuroblastic tumors

Introduction

Peripheral neuroblastic tumors are the most common extra-cranial solid tumor in childhood (Stiller, 2004). They arise from the neuroectodermal cells of the adrenal medulla and sympathetic ganglia and represent a spectrum of cellular maturation and differentiation, which is histologically divided into four categories as per the International Neuroblastoma Pathology Classification (INPC) (Shimada et al., 1999; Peuchmaur et al., 2003). Neuroblastoma is the most primitive, immature and malignant form, histopathologically Schwannian stroma-poor, while ganglioneuroma is a mature benign primitive cell tumor comprised of large ganglion cells considered to result from maturation, and a predominant Schwannian stroma. Ganglioneuroblastomas are heterogenous and can be further divided into intermixed or nodular subtypes. The intermixed subtype is Schwannian stroma-dominant with scattered established or differentiating ganglion cells and prognostically favorable. The nodular subtype is a composite tumor which is Schwannian stroma-rich/dominant and stroma-poor but is characterised by larger nodules, frequently comprised of undifferentiated neuroblasts. These nodular components within ganglioneuroblastoma nodular are considered to be clonally distinct from the remainder of the tumor. Whilst the prognosis of children with ganglioneuroblastoma intermixed is almost invariably very favorable (Shimada et al., 1999; Decarolis et al., 2016) the outcome for children with nodular ganglioneuroblastoma depends upon the characteristics of each individual nodule (grade of differentiation, mitosis-karyorrhexis index (MKI), diploidy) and age of the patient at presentation (Okmatsu et al., 2009; Angelini et al., 2012; Peuchmaur et al., 2003; Shimada et al., 2001). Based on its characteristic a nodule can be defined as favorable or unfavorable, as shown in Table 1. Careful analysis of all the nodules in the histological sample is essential, as the presence of a single unfavourable nodule makes the whole tumor of unfavorable histology and therefore infers a poor prognosis.

Table 1: Histopathological characteristics of ganglioneuroblastoma nodular

Age (months)	Mitosis Karyorrhexis index (MKI)	Differentiation
Favourable nodules		
<18	Low or intermediate	Poorly differentiated or differentiating
18-60	Low	Differentiating
Unfavourable nodules		
<18	High	Undifferentiated
18-60	Intermediate or high	Undifferentiated or poorly differentiated tumor
>60	Any	All tumors

Note: presence of any single unfavourable factor in any single nodule defines unfavourable histology

Key:

Low MKI	<2% or <100 per 5000 cells
Intermediate MKI	2-4% or 100-200 per 5000 cells
High MKI	>4% or 200 per 5000cells

Adapted from Peuchmaur et al. (2003) and Shimada et al. (2001)

Ganglioneuroblastoma nodular represents only a small proportion of peripheral neuroblastic tumors (Angelini et al., 2012; Cohn et al., 2009; Umehra et al., 2000; Okmatsu et al., 2009). Attempts have been made to further inform on the prognosis of this rare type of neuroblastoma. An interesting univariate analysis in the largest INRG cohort identified age, stage M, adrenal primary tumor, *MYCN* amplification and presence of segment chromosomal abnormalities as poor prognostic markers (Angelini et al., 2012). Nevertheless a multi-regression analysis could not be performed given the low number of patients, thus it remains unclear which of these factors plays the more significant role when a mixture of favorable and unfavorable characteristics are present. Certainly, retrospective clinical studies demonstrate a poorer prognosis for children with unfavorable ganglioneuroblastoma nodular than those with ganglioneuroma and ganglioneuroblastoma intermixed (He et al., 2017; Decoralis et al., 2016; Angelini et al., 2012; Cohn et al., 2009; Okmatsu et al., 2009; Umehra et al., 2000). In the context of such a heterogenous tumor and considering the impact that some tumor characteristics have on the prognosis, biopsy is critical in determining the treatment plan for patients with ganglioneuroblastoma nodular.

Here, we highlight how diffusion weighted magnetic resonance imaging (DW-MRI) can inform on tumor biopsy by reporting a case of a five-year old boy with nodular ganglioneuroblastoma.

Case presentation

A four years and eleven month of age boy presented with a twelve-month history of abdominal distension and intermittent right-sided chest discomfort. He was otherwise well and had no other significant symptoms. His medical and surgical history was unremarkable. There was no history of tumor or neuroblastoma in the family.

A chest x-ray showed paucity of gas in the right upper abdomen in keeping with a mass, and faint calcification projected to the right of the T10 vertebral body. An ultrasound of the abdomen demonstrated a large partially calcified abdominal mass and an echogenic area adjacent to the spine. The DW-MRI also revealed a large 15 x 13 x 11cm right-sided retroperitoneal suprarenal multi-locular mass with heterogeneous enhancement, predominately in the superior medial aspect (Figure 1). There was increased signal uptake pre-contrast heterogeneously within the lesion, thought to be related to calcification (Figure 1a&b). There was heterogeneous contrast uptake throughout the lesion (Figure 1c). Interestingly diffusion restriction was limited to one small area in the posteromedial aspect (Figure 1d).

After multidisciplinary discussion, in light of the MRI depiction of a distinct nodule with different restricted characteristics, the decision was made to perform multiple diagnostic biopsies targeting the different areas of the tumor, including the nodule.

Cone-beam computerised tomography (CB-CT) performed at time of biopsy, demonstrated a focus of speckled calcification adjacent to the right-side of the spine (Figure 1e). When CB-CT was fused with the isovolumetric T2-weighted sequence from the preceding MRI, using the bony landmarks to register the fusion, the speckled calcification was confirmed to correspond to the area of restricted diffusion on MRI (Figure 2 a,b,c). Biopsies of the mass were performed under general anaesthetic using a combined ultrasound and CB-CT guidance. Through a posterolateral intercostal, trans-renal, approach a 15G needle was placed into the mass and directed towards the area of restricted diffusion. A repeat CB-CT was performed and fused with the MRI confirming that the correct area was being sampled. Through this needle six core biopsies were performed with a 16G side-notch biopsy needle (Temno Evolution® Biopsy Device, CareFusion, San Diego, California, United States) under ultrasound guidance (Figure 2 d,e,f). The outer needle was then withdrawn slightly and angled anteriorly to allow sampling of the components of the mass which did not show restricted diffusion on MRI (Figure 2 e&f).
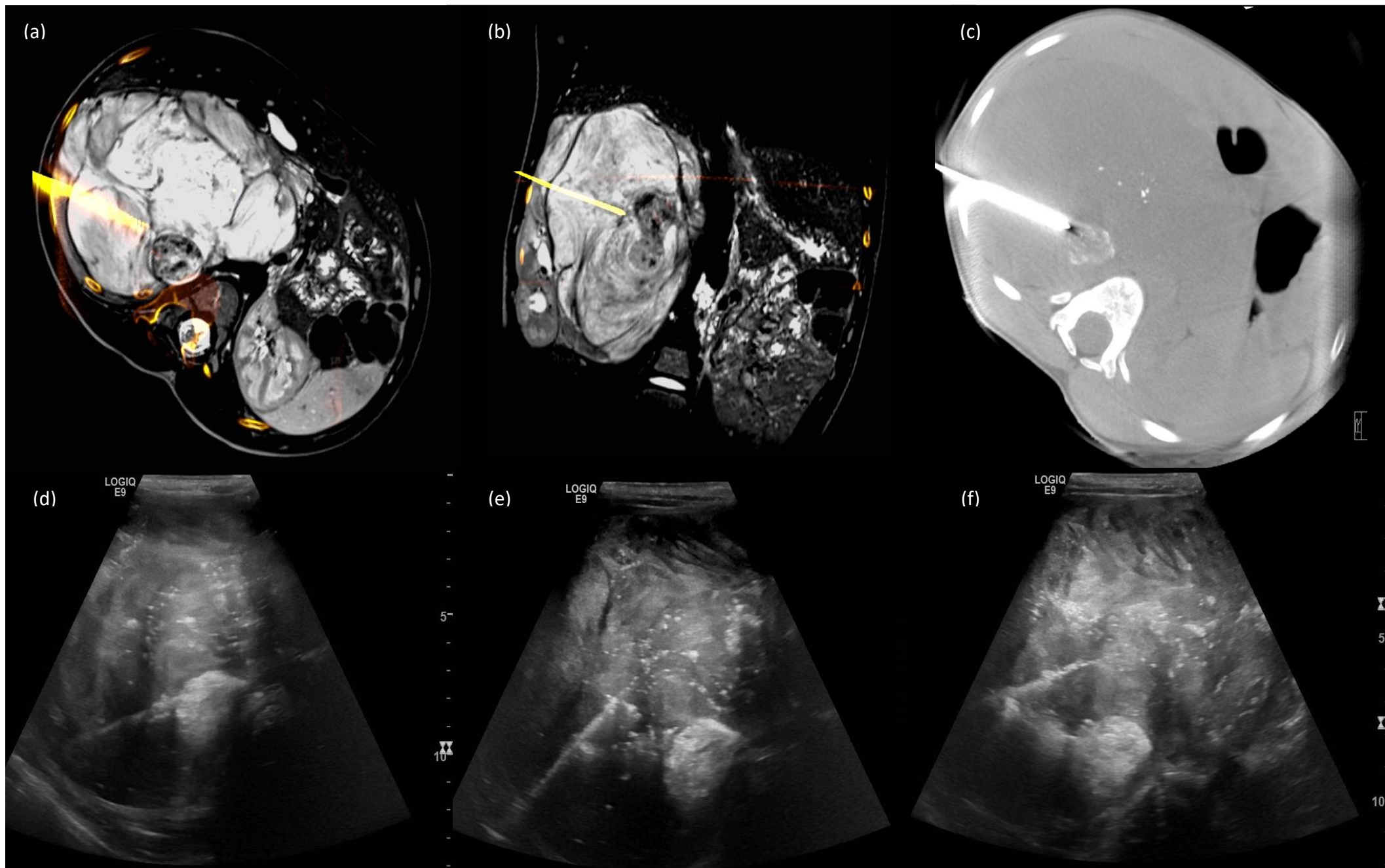
Figure 1: Imaging of lesion
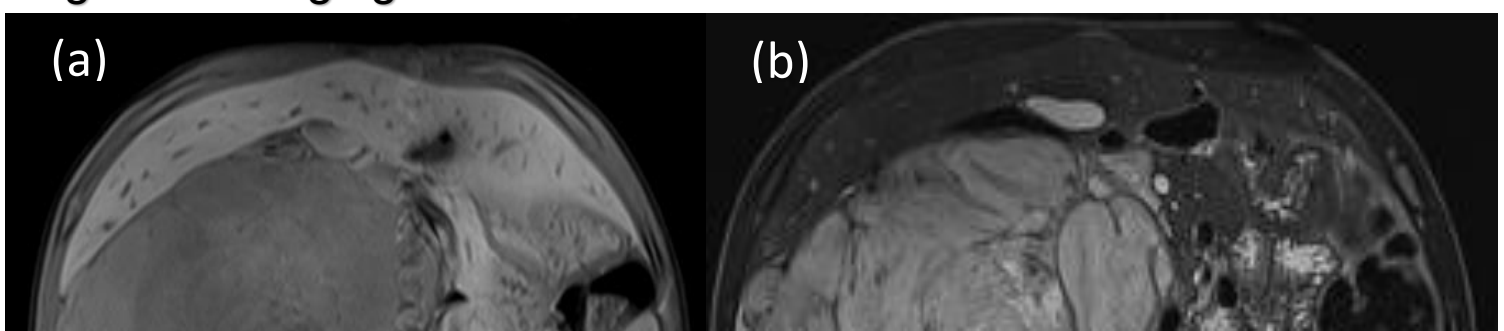
Figure 1

# Figure 2: Biopsy using fused MRI and cone-beam computer tomography and ultrasound guidance

(a) Axial and (b) coronal fused MR and CB-CT images showing biopsy needle at edge of area corresponding to restricted diffusion. (c) CB-CT images of needle at edge of area of speckled calcification. Ultrasound images in transverse plane of biopsy of (d) area of restricted diffusion and (e) (f) areas of non-restricted diffusion

Figure 3: Histopathology
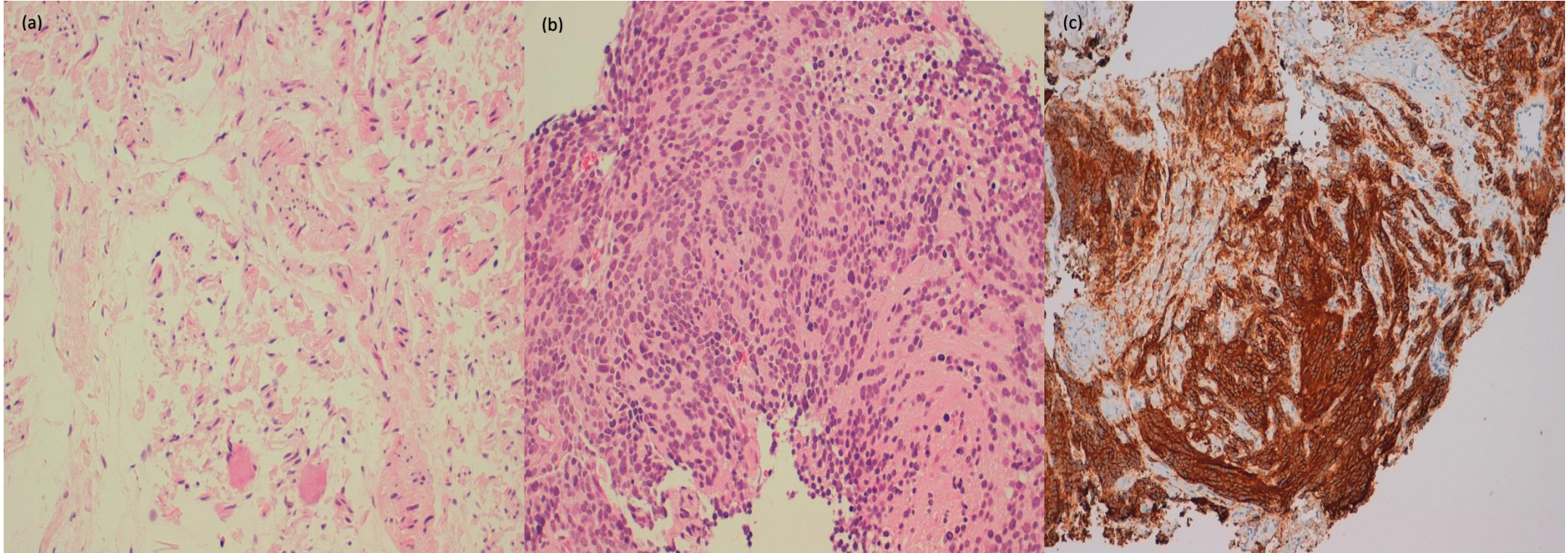
(a) Scattered mature ganglion cells taken from large tumor mass
(b) Small round blue cells consistent with poorly differentiated neuroblastoma taken from nodule
(c) CD56 positive stain of nodule

Histopathology of the core biopsies from the region showing restricted diffusion demonstrated poorly differentiated neuroblasts that were strongly positive for CD56 and NB84 (Figure 3b&c). Biopsy samples from other regions of the mass demonstrated mature fully differentiated ganglion cells (Figure 3a). With the presence of unfavorable histological characteristic of the restricted diffusion nodule and an unfavorable age group (over 5 years of age), a diagnosis of ganglioneuroblastoma nodular-unfavorable subtype was made. Molecular studies confirmed segmental chromosomal aberrations leading to the loss of 11q, gain of 17q, no evidence of *MYCN* amplification, balanced gains of 1p/q and relative gain of *MYCN* within the nodule. There was insufficient DNA to perform molecular studies from the mature ganglion cells component of the tumor.

The diagnostic work-up was completed with negative urinary catecholamines and normal bilateral bone marrow aspirates and trephines. A staging 123-I-MIBG scan with SPECT CT was also performed which showed no evidence of MIBG avid metastatic disease. The abdominal primary tumor demonstrated some focal areas of MIBG uptake but was largely a non-avid lesion. Interestingly, when the CB-CT, DW-MRI and 123-MIBG with SPECT CT were compared, the area of speckled calcification on CB-CT and restricted diffusion on MRI showed only low-grade MIBG uptake (Figure 1e & f). The foci of MIBG avid disease were not particularly restricted on MRI ADC mapping.

As per the International Neuroblastoma Risk Group Staging System (INRGSS) the tumor stage was L2 due to the presence of imaging-defined risk factors, placing the patient in an intermediate risk group according to the Low/Intermediate risk Neuroblastoma European study (LINES) guidelines (Monclair et al., 2009). However, in consultation with the National Advisory Panel for Neuroblastoma in the United Kingdom, the recommended treatment was as per the high risk group with multimodal therapy, given poor survival rates of 30-50% for this group of children with age >60months, presence of segmental chromosomal aberrations and no *MYCN* amplification but unfavorable histology (He et al., 2017; Kohler et al., 2013; Angelini et al., 2012).

The patient started treatment according to the HR-NBL1 SIOPEN protocol. After induction chemotherapy with Rapid COJEC both DW-MRI and 123-I-MIBG scan showed no difference in size or appearances of the large tumor nor of the small undifferentiated nodule. At the time of surgical resection histopathology of the resected tumor showed ganglioneuroma with abundant Schwannian stroma and scattered mature ganglion cells. The DWI-restricted nodule continued to show poorly differentiated neuroblastoma with some areas of differentiating cells and chemotherapy effects including calcification. After discussion with his parents it was

decided not to proceed with high dose chemotherapy but the patient continued to receive local control treatment (radiotherapy) followed by cis-retinoic acid. Given that high dose chemotherapy with Busulfan and Melphalan had not been given, anti-GD2 antibody treatment (dinituximab-beta) was also not administered. There have been expected side effects of treatment, including bilateral mild to moderate high frequency hearing loss (Brock Grade II, Boston Grade III) and right sided nephrectomy. The patient remains alive at the time of this report, 2.5 years after diagnosis.

Discussion

As nodular ganglioneuroblastoma remains a disease with low patient numbers, a clear pathway for optimal diagnosis and treatment has not yet been established. The INPC clearly differentiates this histological subtype of peripheral neuroblastic tumors into favorable and unfavorable subtypes, which correlate with a very different prognosis in retrospective clinical studies. Though the overall 5 year event free survival (EFS) is 53% (Angelini et al., 2012), when grouped by subtype the EFS is significantly different: children with favorable GNB-nodular demonstrate a 5 year EFS of 87% or an 8 year EFS of 91% vs those with unfavorable GNB-nodular who have a 5 year EFS of 45% or an 8 year EFS of 33% (Angelini et al., 2012; Peuchmaur et al., 2003). Unfavorable histology, grade of differentiation, *MYCN* amplification, DNA index <= 1 and chromosomal aberrations have been reported to correlate with poor EFS in nodular ganglioneuroblastoma in a univariate analysis (Angelini et al., 2012). Further, the SIOPEN NB2009 study demonstrated that children more than five years of age with localized, *MYCN* not amplified but unfavorable histology disease had a five year EFS of 35% in comparison to 72% for children in the 18 to 60 month-age group. Also children who are older than 5 years with undifferentiated or poorly differentiated tumor histology are at higher risk of relapse, similar to those children with metastatic disease or *MYCN* amplification (Kohler et al, 2013). Thus, accurate biopsies which represent the heterogeneity inherent in these tumors are of paramount importance. In our case the identification of the unrestricted nodule could have been missed if DWI sequencing were not performed, and a biopsy of a more peripheral, easily accessible area would have been performed with the risk of a misdiagnosis of ganglioneuroma.

Several authors have investigated the role of DW-MRI in predicting histopathology of peripheral neuroblastic tumors and there appears to be general consensus that apparent diffusion coefficient (ADC) is significantly lower for neuroblastoma and nodular ganglioneuroblastoma when compared to ganglioneuroma and intermixed ganglioneuroblastoma, and this could be used as an imaging marker for histological sampling (Serin et al., 2016; Gahr et al., 2011; Wen et al., 2017; Neubauer et al., 2017). The cut off

ADC varies between studies and a consensus has not yet been established. ADC below 0.93×10^{-3} mm^2/s with a sensitivity of 74% and specificity of 67% for detecting neuroblastoma (Serin et al, 2016), ADC below 1.1×10^{-3} mm^2/s for detecting neuroblastoma (Gahr et al. (2011), ADC below 1.165×10^{-3} mm^2/s with sensitivity of 94% and specificity of 100% for detecting neuroblastoma and nodular ganglioneuroblastoma (Wen et al., 2017) or ADC below 1.0×10^{-3} mm^2/s for detecting neuroblastoma and nodular ganglioneuroblastoma (Neubauer et al., 2017) have all been suggested. The number of patients with nodular ganglioneuroblastoma are low in these small studies and there had been favorable and unfavorable histology tumors included (Wen et al., 2017).

This case report is novel, as we have used DW-MRI to identify and guide biopsy of a poorly differentiated nodule within nodular ganglioneuroblastoma, which to our knowledge has not been reported before. Biopsy of lesions that are only visible on MRI can be performed under MRI guidance but this is challenging, as it requires compatible needles and often long procedure times. An alternative method involves fusing the MRI with CB-CT images, as done in this case. This fusion image can then be used as an overlay for fluoroscopic guidance of the biopsy needle to the target in a heterogeneous lesion (Thakor et al, 2016). Positron emission tomography-computed tomography (PET-CT)/ultrasound fusion imaging guidance is another technique that may be useful for PET-positive lesions (Paparo et al., 2014). Takeda et al. (2018) describe a case report using fluorodeoxyglucose PET-CT for identification and biopsy a favorable histology neuroblastic nodule within nodular ganglioneuroblastoma.

Conclusion

In summary, this case report highlights the importance of using DW-MRI for identifying and guiding biopsy of a neuroblastic nodule in nodular ganglioneuroblastoma, which can significantly impact prognosis and treatment. With the increasing acceptance of DW-MRI as a method of radiologically distinguishing between different histological types of peripheral neuroblastic tumors, this case report suggests an additional benefit for DW-MRI in nodular ganglioneuroblastoma, as it may also help identify areas to target for biopsy and the use of fused MRI and CB-CT images to help guide such procedure.

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