Scientific Research Publishing

Open Journal of Pathology, 2021, 11, 100-109
https://www.scirp.org/journal/ojpathology
ISSN Online: 2164-6783
ISSN Print: 2164-6775

A Case of Ewing's Sarcoma Arising in the Cervical Spine with an Elevation of Serum ProGRP

Ryogo Aoki[1], Yoshikazu Ikoma[2], Asuka Ohashi[1], Riyoko Niwa[1], Fumumasa Etori[1], Masashi Matsuyama[1], Akane Onogi[1], Naoki Watanabe[1], Takuji Tanaka[1]*

[1]Departments of Diagnostic Pathology (DDP) & Research Center of Diagnostic Pathology (RC-DiP), Gifu Municipal Hospital, Gifu, Japan
[2]Departments of Hematology, Gifu Municipal Hospital, Gifu, Japan
Email: *takutt@gmhosp.gifu.gifu.jp

How to cite this paper: Aoki, R., Ikoma, Y., Ohashi, A., Niwa, R., Etori, F., Matsuyama, M., Onogi, A., Watanabe, N. and Tanaka, T. (2021) A Case of Ewing's Sarcoma Arising in the Cervical Spine with an Elevation of Serum ProGRP. *Open Journal of Pathology*, **11**, 100-109.
https://doi.org/10.4236/ojpathology.2021.114009

Received: August 6, 2021
Accepted: September 3, 2021
Published: September 6, 2021

Abstract

We experienced a case of small, round-cell malignant neoplasm diagnosed by touch smear cytology and histopathology when an open biopsy was performed in a 50-year-old Japanese woman. She was suspected of having a cervical spine tumor after surgery for cervical spine foraminal stenosis. After consent, the cervical spine tumor histologically diagnosed by an open biopsy was confirmed to be Ewing sarcoma (EWS) by genetic testing. EWS belongs to a group of small, round-cell tumors that are morphologically similar and often difficult to differentiate. After the open biopsy, the present patient received radiotherapy, and her plasma level of Pro-Gastrin-Releasing-Peptide was decreased (217.2 pg/ml before surgery to 30.3 pg/ml; reference value: 0 - 80 pg/ml). We herein report the process for making the final diagnosis by focusing on the intraoperative cytology, histopathology, and immunohistochemical findings. Our diagnosis was validated by karyotyping and a fluorescence *in-situ* hybridization analysis.

Keywords

Ewing's Sarcoma, Small, Round-Cell Tumors, ProGRP, Touch Smear Cytology, Intra-Operative Pathological Diagnosis, Immunohistochemistry

1. Introduction

A small, round-cell sarcoma, Ewing's sarcoma (EWS), occurs mainly in bone and soft tissue. EWS is a rare disease with an annual incidence of 1 in 1 million

[1]. The peak incidence is between 15 and 20 years old, with about 80% of cases occurring under 18 years old, and only approximately 1% of cases occurring over 40 years old [1] [2] [3]. The prognosis for EWS depends on the presence or absence of metastases, with a survival rate of more than 70% for patients without metastases at the time of the diagnosis [4], and a 5-year survival rate of less than 30% for patients with metastases [5].

Morphologically, EWS belongs to a group of small, round-cell tumors that includes several histologic types of tumors, such as desmoplastic small, round-cell tumor, synovial sarcoma, rhabdomyosarcoma, neuroblastoma, Wilms tumor, malignant lymphoma, and small-cell carcinoma. These tumors are difficult to differentiate morphologically and are often difficult to diagnose as well. In 2020 WHO classification of bone and soft tissue tumors [6], EWS is now classified as undifferentiated small, round-cell sarcoma of the bone and soft tissue, along with round-cell sarcoma with EWSR1-non-ETS fusions, CIC-rearranged sarcoma, and sarcoma with BCOR genetic alterations [6]. For the definitive diagnosis of these tumors, the detection of fusion genes is necessary [6].

We herein report a case of EWS that developed in the cervical spine of a 50-year-old Japanese woman who was originally diagnosed with cervical spine foraminal stenosis. After surgery for cervical spine foraminal stenosis, the symptoms did not disappear. Therefore, re-operation for an open biopsy to histopathologically diagnose what was suspected of being a cervical spine tumor based on computed tomography (CT) findings was performed. During the re-operation, we diagnosed the imprint with a small, round-cell malignant neoplasm based on the findings of smear cytology, histopathology, and immunohistochemistry, and finally confirmed it to be EWS by genetic testing.

2. Case Presentation

The patient was a 50-year-old Japanese woman who complained of pain in the right scapula and upper limbs and paralysis in the same area for 4 months. Informed consent was obtained from the patient for this case report. Posterior cervical spine decompression fusion was performed because C4-5 cervical spine right foraminal stenosis was suspected on magnetic resonance imaging (MRI). However, her symptoms did not disappear even after the operation. Furthermore, postoperative follow-up CT indicated an osteolytic lesion in the C5 cervical spine that had enlarged by three weeks after the operation (**Figure 1(a)**, **Figure 1(b)**). In addition, elevated levels of serum pro-gastrin-releasing-peptide (ProGRP; 217.2 pg/ml; reference value 0 - 80 pg/ml) were noted. However, the serum level (13.0 ng/ml) of neuro-specific enolase (NSE) was within the reference range (0 - 16.3 ng/ml).

Fluorodeoxyglucose-positron emission tomography (^{18}F-FDG-PET)/CT revealed an increased uptake of ^{18}F-FDG in the osteolytic lesion of the C5 cervical spine (**Figure 2**), suggesting the presence of a malignant tumor. Therefore, an intraoperative frozen section diagnosis was performed to determine whether the C5

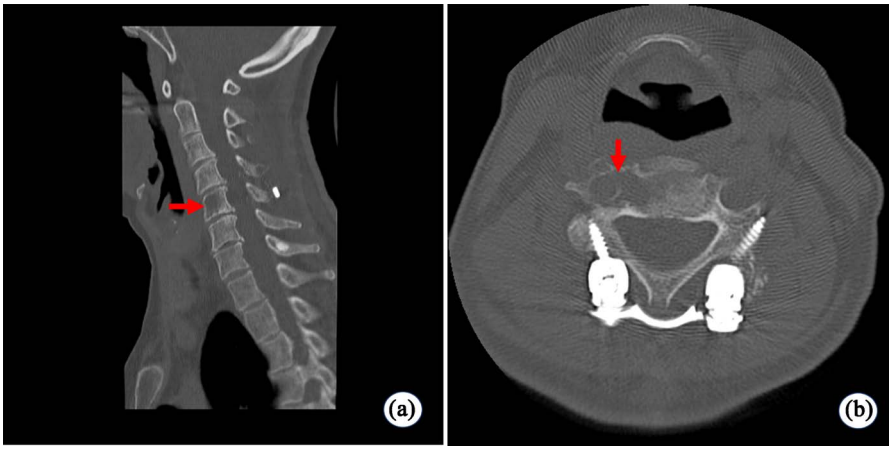

Figure 1. Follow-up CT performed at three weeks after posterior cervical spine decompression and fusion. Note the osteolytic lesion (arrow) in the C5 vertebra on the (a) sagittal and (b) axial views of the cervical spine. The osteolytic lesion was enlarged before the surgery for cervical posterior decompression and fusion, suggesting a malignant tumor.

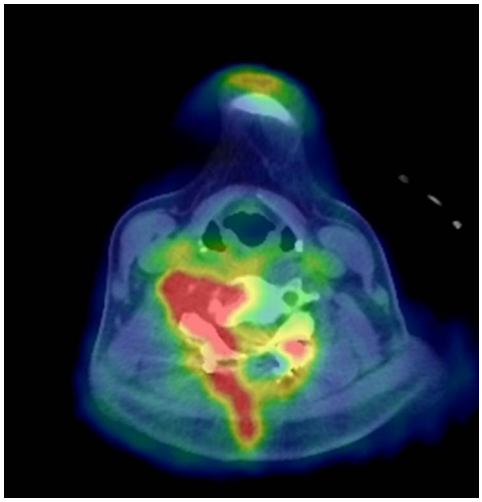

Figure 2. An axial fused positron-emission tomography/computed tomography (PET/CT) image shows an increase in the ^{18}F-FDG uptake in an osteolytic lesion at the right side of the C5 vertebral body and its paravertebral region.

cervical spine lesion was benign or malignant. Intraoperative materials were processed for touch smear cytology and histopathology. In addition, a chromosome analysis including karyotyping (G-banding) and a translocation analysis by fluorescence *in-situ* hybridization (FISH) was performed to validate the diagnosis made by morphology, immunohistochemistry, and karyotyping.

Intraoperative touch smear cytology and the histological diagnosis

When cytologic specimens of the osteolytic lesion of the C5 cervical spine were stained with the Papanicolaou and Giemsa methods, small, round tumor cells with an extremely high nuclear-cytoplasmic (N/C) ratio were present in small clusters and/or isolated. The nuclear chromatin of the tumor cells was dense and granularly fine. Their nucleoli were inconspicuous. In some areas, nuclear crush artifacts were observed (**Figures 3(a)-(d)**). We cytologically diagnosed the entity as a small,

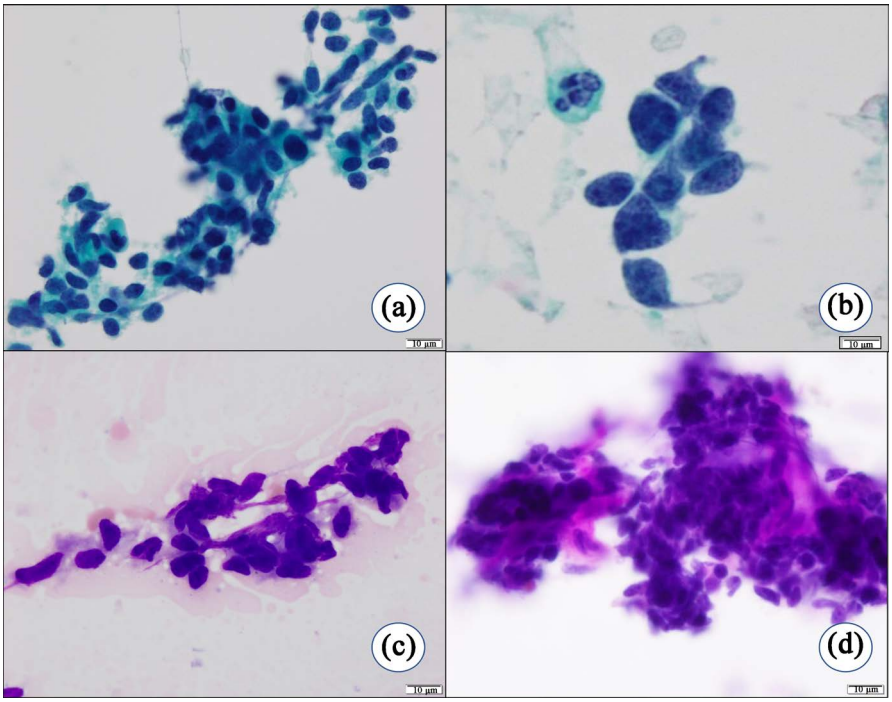

Figure 3. Intraoperative touch smear cytology made from a cervical spine lesion. (a)-(c) Small round tumor cells with extremely high N/C ratio are present in small clusters. The nuclear chromatin of the tumor cells is dense and granularly fine. Their nucleoli are inconspicuous. (d) The tumor cells are positive for PAS staining in their cytoplasm. (a) and (b) Papanicolaou stain, (c) May-Gruenwald Giemsa Stain, (d) Periodic acid-Schiff reaction, bars = 10 μm.

round-cell malignant neoplasm, possibly small-cell carcinoma.

Frozen section specimens stained with hematoxylin and eosin (H & E) showed similar findings of diffuse growth of small round tumor cells with a high N/C ratio, and in some areas nuclear crush artifacts were present (data not shown). We again suspected a small, round-cell malignant neoplasm, including small-cell carcinoma and malignant lymphoma. To confirm this diagnosis, we added immunohistochemical and PAS staining to the permanent specimens.

The histopathological examination of the cervical spine lesions

Formalin-fixed paraffin-embedded (FFPE) permanent specimens were stained with H&E. Growth and invasion of small, round tumor cells with a high N/C ratio were observed with hemorrhaging (**Figure 4(a)**). The histological features were similar to those of the frozen section specimens used for the intraoperative pathological diagnosis. The cytoplasm of the tumor cells was positive on PAS staining (**Figure 4(b)**), suggesting that the tumor was not small-cell carcinoma. Immunohistochemically, the tumor cell membrane was positive for CD99 (**Figure 4(c)**) and CD56 (**Figure 4(d)**), but negative for synaptophysin, chromogranin A, AE1/AE3, and 34βE12. The MIB-1-positive index of the tumor cells was approximately 20%. We ultimately suspected EWS or CD99-positive lymphoblastic lymphoma.

Karyotyping by G-banding

G-banded karyotyping was performed to determine chromosomal abnormalities. The karyotype showed chromosomal aberration at t (11;22) (q24;q12) (Figure 5), suggesting EWS.

*An EWSR*1 22*q*12 *translocation analysis by the FISH method*

An EWSR1 22q12 translocation analysis by FISH was performed for EWSR1

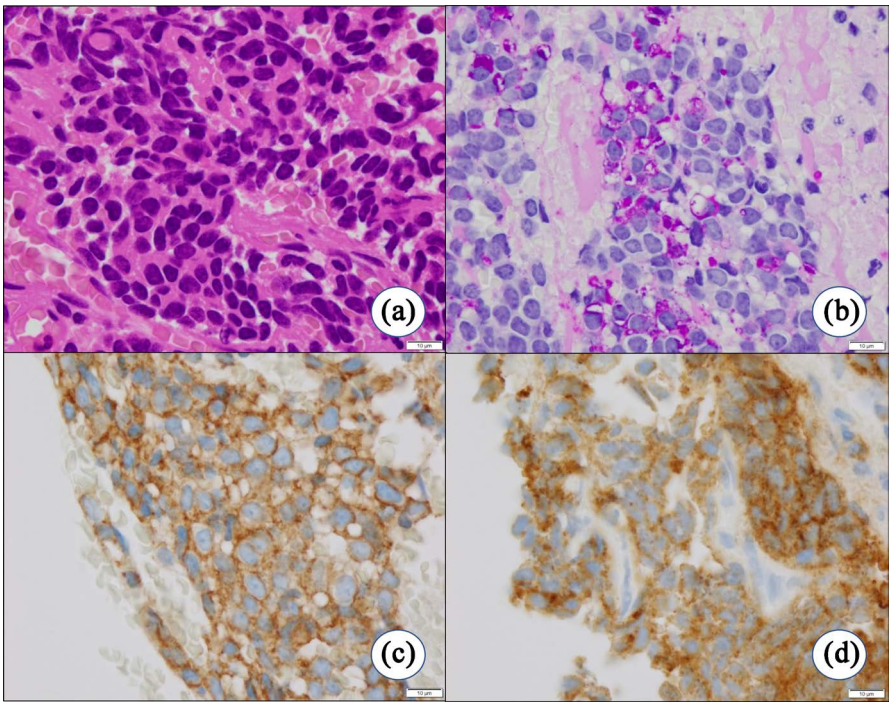

Figure 4. Histopathology and immunohistochemistry of a cervical spine lesion obtained during re-operation for an open biopsy. (a) The lesion consists of small tumor cells with a round shape and high N/C ratio. (b) The PAS stain shows a positive cytoplasmic reaction. Tumor cells show a membranous positive reaction against (c) CD99 and (d) CD56. (a) Hematoxylin and eosin (H&E) stain, bar = 10 μm, (b) Periodic acid-Schiff (PAS) reaction, (c) CD99 immunohistochemistry, and (d) CD56 immunohistochemistry. Bars = 10 μm.

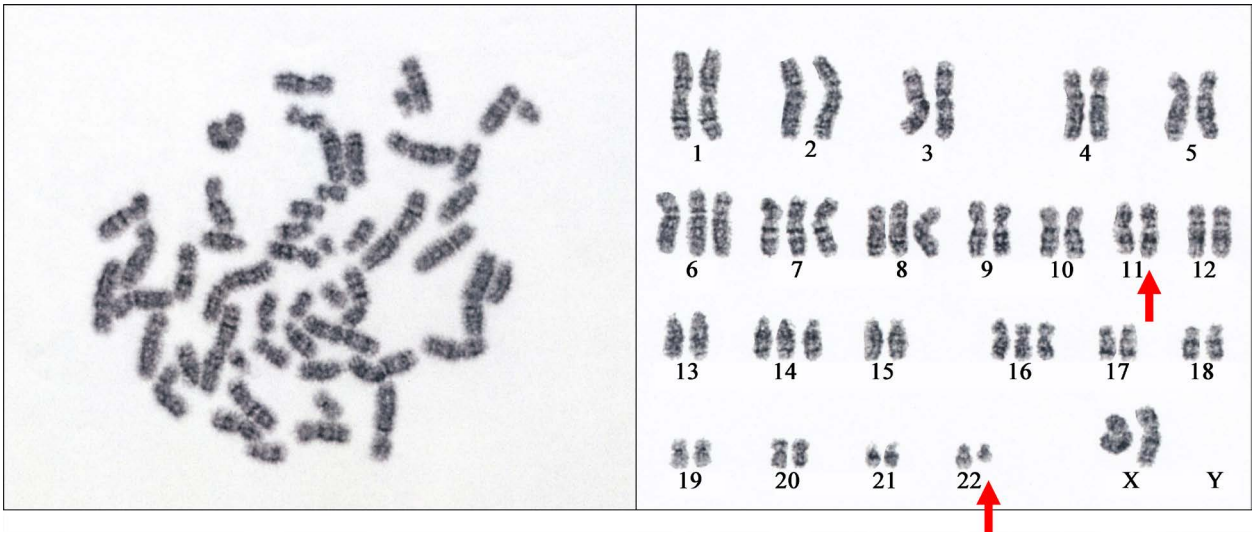

Figure 5. G-banded karyotyping shows chromosomal aberrations (arrows) in t (11; 22Xq24; q12).

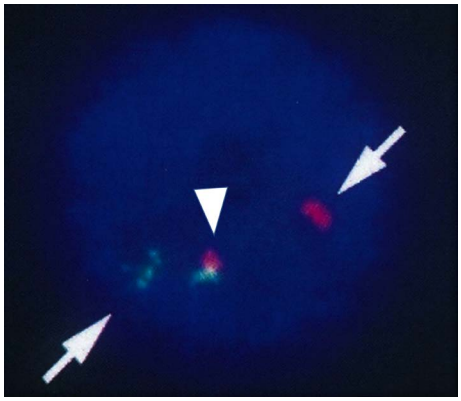

Figure 6. A dual-color break-apart FISH assay for the analysis of EWSR1 fusion using the probes (EWSR1-3' and EWSR1+5') for EWSR1 (22q12, Abbott Molecular Inc., IL, USA). The 5' and 3' ends are labeled with FITC and Texas Red, respectively. This image shows the fusion (arrow-head) and, red (arrow), and green (arrow) signal patterns indicative of a rearrangement of one copy of the EWSR1 region.

rearrangement (arrows in **Figure 6**). A translocation analysis using the EWSR1 probe detected a split signal in the 22q12 region (arrow-head in **Figure 6**).

Based on the results described above, our final diagnosis of this case was EWS with an EWSR1-FLI1 fusion gene by t(11; 22) (q24; q12): Stage II, T1N0M0, Undifferentiated. Radiotherapy (a total of 54.2 Gy) after the open biopsy resulted in a decrease of the serum level (30.3 pg/ml) of ProGRP (the normal rage, 0-80 pg/ml). The patient then followed by the VDC-IE chemotherapy: a total of 14 cycles with 2 weeks of interval; VDC consisted of cyclophosphamide (1,200 mg/m^2), doxorubicin (75 mg/m^2), and vincristine (1.5 mg/m^2); and IE included ifosfamide (9,000 mg/m^2) and etoposide (500 mg/m^2). During the chemotherapy, the patient had slight digestive symptoms such as nausea or vomiting. Although the tumor did not completely regress at one year after her diagnosis of EWS, she showed an uneventful clinical course without metastasis.

3. Discussion

Most cases of EWS occur in the upper and lower limbs and pelvis [7], and vertebral occurrence is low with an incidence of 9.8% [8]. The distribution in such cases has been reported to be to the sacral spine (53.2%), lumbar spine (25%), thoracic spine (10.5%), and cervical spine (3.2%) [8]. Soft-tissue EWS occurs mainly in the paraspinal region and deep soft tissues of the proximal extremities. However, it is difficult to determine whether the primary lesion is bone or soft tissue because of the bone involvement of spinal tumors. The present case, where EWS occurred in the cervical spine of a 50-year-old patient, is a rare one considering the age and region affected. Whether the primary site was the cervical spine or soft tissue surrounding the cervical spine was unclear, but PET-CT showed the accumulation of FDG throughout the cervical spine, suggesting that the primary site was the cervical spine.

Recent immunostaining and ultrastructural studies have revealed that EWS is

a tumor derived from the neuroectodermal lobe. The characteristic translocations t (11; 22) (q24; q12) and shared genetic aberrations with chimeric genes of ETS family genes such as EWSR1 and FLI1 suggest that EWS has the same cellular origin as peripheral primitive neuroectodermal tumor (pPNET), although their histopathology is different [9]. EWS and pPNET, both showing some neuroectodermal traits, are considered to lie at the poorly and well-differentiated ends of the round-cell sarcoma spectrum, respectively [9] [10]. On the EWS side of the histopathological spectrum, the tumor cells consist of small tumor cells with round to oval nuclei, well-defined nuclear membranes, small nucleoli, and poorly defined cytoplasm with indistinct borders [9]. On the pPNET side of the pathomorphologic spectrum, tumor cells have more abundant eosinophilic cytoplasm and well-defined nucleoli [6] [11]. In more differentiated tumors, many rosette structures are observed, while intracellular glycogen is present in many undifferentiated cases [6] [11]. In less than half of EWS/pPNETs, rosette formation by tumor cells with PAS-positive cytoplasm is observed.

The tumor cells in the present case were morphologically and genetically diagnosed as EWS, because they contained the EWSR1-FLI1 fusion gene by t (11; 22) (q24; q12) according to G-banded karyotyping and an EWSR1 22q12 translocation analysis by FISH. The intraoperative cytological diagnosis of this case was a small, round-cell malignant neoplasm, possibly small-cell carcinoma. On the imprint cytological specimens, we noted no rosette formation by tumor cells, but nuclear crush artifacts were seen.

Based on the above findings as well as the high serum level of ProGRP, we suspected small cell carcinoma. However, PAS reaction was positive in the cytoplasm of tumor cells on the tissue specimens where there was no rosettes formation. Therefore, we attempted to stain the PAS reaction on the Papanicolaou-stained stamp slides after removing the color, resulting in the observation of cytoplasmic positive staining in the tumor cells. These findings indicated that the tumor was on the EWS side (poorly differentiated) and did not differentiate into the neuroectodermal lobe.

Morphologically, EWS belongs to the small, round-cell tumor group, which contains several types of malignant tumors; however, these lesions are sometimes difficult to differentiate morphologically. For conclusive differentiation, immunohistochemistry using several antibodies is useful and practical [12]. CD99 is a useful for diagnosing EWS, as strong membrane positivity of CD99 is noted in EWS tumor cells, regardless of the degree of differentiation. However, CD99 is also positive in many other round tumors, such as synovial sarcoma, Merkel cell tumor, lymphoblastic lymphoma, rhabdomyosarcoma, mesenchymal chondrosarcoma, solitary fibrous tumor, and desmoplastic round-cell tumor [13] [14], although it is often negative in central PNETs [15]. Interestingly, NKX2.2 immunohistochemistry has been reported to be useful for diagnosing EWS [16].

Another interesting finding is the elevation of the serum level of ProGRP, a

tumor marker for small-cell carcinoma. After the open biopsy and radiotherapy, the level decreased to the normal range, suggesting that the tumor cells were producing ProGRP. In 2015, Yamaguchi *et al.* [17] first reported the usefulness of ProGRP as a tumor marker for EWS. They subsequently reported that serum ProGRP levels were high in 8 of 16 patients with EWS, and the serum NSE level was elevated in 14 of 16 EWS patients [18]. Furthermore, during treatment. the ProGRP level correlated with the tumor volume, indicating that ProGRP is a useful marker for both the diagnosis and evaluation of the therapeutic effects of EWS [18]. Plasma NSE levels in our patient were within the normal range, while the serum ProGRP level was elevated. This may suggest that the serum level of NSE in this case reflected the poor differentiation of tumor cells into nerves.

4. Conclusion

When cytologically and histopathologically diagnosing, small round-cell tumors, it is often difficult to make a morphological differential diagnosis. To validate the morphological diagnosis and make a definitive diagnosis of small, round-cell neoplasms, certain genetic analyses are necessary, as described in this case.

Acknowledgements

The authors would like to thank Ms. Toshie Naraki for her assistance in the manuscript review process.

Authors' Contributions

R.A. collected the data, made the cytological diagnosis, and wrote the manuscript. Y.I. performed the G-banded karyotyping and FISH analysis and treated the patient. A.O., R.N., F.E., M.M., and Ak.O. made the cytological diagnosis. Ak.O. and N.W. made the pathological diagnosis. T.T. made substantial contributions to the interpretation of data and manuscript review. All authors read and approved the final manuscript.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

References

[1] Hesla, A.C., Papakonstantinou, A. and Tsagkozis, P. (2021) Current Status of Management and Outcome for Patients with Ewing Sarcoma. *Cancers*, **13**, 1202. https://doi.org/10.3390/cancers13061202

[2] Khan, S., Abid, Z., Haider, G., Bukhari, N., Zehra, D., Hashmi, M., Abid, M. and Ibrahim, U. (2021) Incidence of Ewing's Sarcoma in Different Age Groups, Their Associated Features, and Its Correlation With Primary Care Interval. *Cureus*, **13**, e13986. https://doi.org/10.7759/cureus.13986

[3] Cotterill, S.J., Ahrens, S., Paulussen, M., Jurgens, H.F., Voute, P.A., Gadner, H. and Craft, A.W. (2000) Prognostic Factors in Ewing's Tumor of Bone: Analysis of 975

Patients from the European Intergroup Cooperative Ewing's Sarcoma Study Group. *Journal of Clinical Oncology*, **18**, 3108-3114. https://doi.org/10.1200/JCO.2000.18.17.3108

[4] Esiashvili, N., Goodman, M. and Marcus Jr., R.B. (2008) Changes in Incidence and Survival of Ewing Sarcoma Patients over the Past 3 Decades: Surveillance Epidemiology and End Results Data. *Journal of Pediatric Hematology/Oncology*, **30**, 425-430. https://doi.org/10.1097/MPH.0b013e31816e22f3

[5] Stahl, M., Ranft, A., Paulussen, M., Bolling, T., Vieth, V., Bielack, S., Gortitz, I., Braun-Munzinger, G., Hardes, J., Jurgens, H. and Dirksen, U. (2011) Risk of Recurrence and Survival after Relapse in Patients with Ewing Sarcoma. *Pediatric Blood & Cancer*, **57**, 549-553. https://doi.org/10.1002/pbc.23040

[6] de Alava, E., Lessnick, S.L. and Stamenkovic, I. (2020) Undifferentiated Small, Round-Cell Sarcomas of Bone and Soft Tissue. Ewing Sarcoma. In: Bridge, J.A., Ed., *WHO Classification of Tumours: Soft Tissue and Bone Tumours*, International Agency for Research on Cancer (IARC), Lyon.

[7] Paulussen, M., Ahrens, S., Dunst, J., Winkelmann, W., Exner, G.U., Kotz, R., Amann, G., Dockhorn-Dworniczak, B., Harms, D., Muller-Weihrich, S., Welte, K., Kornhuber, B., Janka-Schaub, G., Gobel, U., Treuner, J., Voute, P.A., Zoubek, A., Gadner, H. and Jurgens, H. (2001) Localized Ewing Tumor of Bone: Final Results of the Cooperative Ewing's Sarcoma Study CESS 86. *Journal of Clinical Oncology*, **19**, 1818-1829. https://doi.org/10.1200/JCO.2001.19.6.1818

[8] Ilaslan, H., Sundaram, M., Unni, K.K. and Dekutoski, M.B. (2004) Primary Ewing's Sarcoma of the Vertebral Column. *Skeletal Radiology*, **33**, 506-513. https://doi.org/10.1007/s00256-004-0810-x

[9] Ushigome, S., Machinami, R. and Sorensen, P.H. (2002) Ewing Sarcoma/Primitive Neuroectodermal Tumour (PNET). In: Fletcher, C.D.M., Unni, K.K., Mertens, F., Eds., *WHO Classification of Tumours: Pathology and Genetics of Tumours of Soft Tissue and Bone*, IARC Press, Lyon, 298-300.

[10] Heymann, D. (2014) Bone Cancer: Primary Bone Cancers and Bone Metastases. Academic Press, Cambridge.

[11] Welsch, T., Mechtersheimer, G., Aulmann, S., Mueller S.A., Buechler, M.W., Schmidt, J. and Kienle, P. (2006) Huge Primitive Neuroectodermal Tumor of the Pancreas: Report of a Case and Review of the Literature. *World Journal of Gastroenterology*, **12**, 6070-6073. https://doi.org/10.3748/wjg.v12.i37.6070

[12] Ikeda, K. and Tsuta, K. (2016) Effusion Cytomorphology of Small, Round-Cell Tumors. *Journal of Cytology*, **33**, 85-92. https://doi.org/10.4103/0970-9371.182529

[13] Riggi, N. and Stamenkovic, I. (2007) The Biology of Ewing Sarcoma. *Cancer Letters*, **254**, 1-10. https://doi.org/10.1016/j.canlet.2006.12.009

[14] Baldauf, M.C., Orth, M.F., Dallmayer, M., Marchetto, A., Gerke, J.S., Rubio, R.A., Kiran, M.M., Musa, J., Knott, M.M.L., Ohmura, S., Li, J., Akpolat, N., Akatli, A.N., Ozen, O., Dirksen, U., Hartmann, W., de Alava, E., Baumhoer, D., Sannino, G., Kirchner, T. and Grunewald, T.G.P. (2018) Robust Diagnosis of Ewing Sarcoma by Immunohistochemical Detection of Super-Enhancer-Driven EWSR1-ETS Targets. *Oncotarget*, **9**, 1587-1601. https://doi.org/10.18632/oncotarget.20098

[15] Vural, C., Uluoglu, O., Akyurek, N., Oguz, A. and Karadeniz, C. (2011) The Evaluation of CD99 Immunoreactivity and EWS/FLI1 Translocation by Fluorescence *in Situ* Hybridization in Central PNETs and Ewing's Sarcoma Family of Tumors. *Pathology and Oncology Research*, **17**, 619-625. https://doi.org/10.1007/s12253-010-9358-3

[16] McCuiston, A. and Bishop, J.A. (2018) Usefulness of NKX2.2 Immunohistochemistry for Distinguishing Ewing Sarcoma from Other Sinonasal Small Round Blue Cell Tumors. *Head and Neck Pathology*, **12**, 89-94. https://doi.org/10.1007/s12105-017-0830-1

[17] Yamaguchi, K., Katagiri, H., Takahashi, M., Ishida, Y., Ono, A., Takahashi, T., Ohshima, K., Mochizuki, T., Urakami, K., Muramatsu, K., Kameya, T., Ito, I. and Nakajima, T. (2015) ProGRP Is a Possible Tumor Marker for Patients with Ewing Sarcoma. *Biomedical Research*, **36**, 273-277. https://doi.org/10.2220/biomedres.36.273

[18] Honda, Y., Katagiri, H., Takahashi, M., Murata, H., Wasa, J., Hosaka, S., Ishida, Y., Ito, I., Muramatsu, K., Mochizuki, T., Matsuyama, Y. and Yamaguchi, K. (2019) Pro-Gastrin-Releasing Peptide as a Marker for the Ewing Sarcoma Family of Tumors. *International Journal of Clinical Oncology*, **24**, 1468-1478. https://doi.org/10.1007/s10147-019-01492-0