Original Article

Sub-categorization of Pediatric Small Round Blue Cell Tumors using Immunohistochemistry

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Abstract

Introduction: The category of Small Round Blue Cell Tumors includes neoplasms that are undifferentiated and contain similar-looking growth of small round blue cells having bigger nuclear size as compared to the cytoplasm (high N/C). It includes Non-Hodgkins Lymphoma, Synovial Sarcoma, Ewings Sarcoma/ Primitive Neuroectodermal Tumor, Rhabdomyosarcoma, Hepatoblastoma, Retinoblastoma, Neuroblastoma, Neuroendocrine carcinoma, Nasopharyngeal Carcinoma, Desmoplastic Small Round Cell Tumor, Dysgerminoma, and Wilm's tumor. Immunohistochemistry can be very helpful in the accurate diagnosis of this diverse group of tumors.

Objective: The objective of the study is to sub-categorize pediatric malignant small round blue cell tumors using immunohistochemistry.

Study design: Descriptive cross-sectional study

Setting: Meezan private lab, Faisalabad, Pakistan

Duration of study: 4 years, from January 2017 to December 2020.

Sample size: 46 cases.

Sampling technique: Non-probability purposive sampling

Materials and Methods: 46 cases, which fulfilled the inclusion and exclusion criteria were selected for the study. All these cases were subjected to immunohistochemistry. The IHC technique used was based on the Peroxidase anti-peroxidase (PAP) method. Based on site and morphological clues, initially Leukocyte common antigen (LCA), Myogenin, Cytokeratin (CK), Desmin, Chromogranin, Neuron Specific Enolase (NSE), S-100, Smooth Muscle Actin (SMA), and CD99 were used. Further immune stains panels were used afterward, as and when needed like CD20, CD3, CD30, BCL2, CD117, Ki-67, Tdt, Synaptophysin, SMA, CD56, Melan A, HMB45, and WT1.

Results: Among all the malignant Small Round Cell Tumors, Rhabdomyosarcoma was the highest in frequency i.e. 8 (17.4%), followed by Ewing's sarcoma/PNET 7(15.2%). Both Diffuse Large B cell lymphoma and Neuroblastoma were 5 each in number (10.9%). Non-Hodgkins lymphoma as a whole was 13 (28.2%), including 5 cases (10.9%) of diffuse large B cell lymphoma, 4 cases (8.7%) of T lymphoblastic lymphoma, 3 cases (6.5%) of Burkitt's lymphoma, and only 1 case (2.2%) of NK/T cell lymphoma.

Conclusion: Immunohistochemistry is an essential tool for accurate sub-categorization of pediatric small round blue cell tumors.

Keywords: Malignant small round blue cell tumor, MSRBCT, pediatric Round Blue cell tumor, Immuno-histochemistry.

Introduction

Round Blue Cell Tumor (RBCT) is a diagnosis that can be a lifesaver for the working pathologist since it gives a clue to the malignant nature of the neoplasm, but practically speaking, it becomes a dilemma for the treating physician. Most of the time, it leads to a series of investigations and consultations, which not only increases the burden on medical resources but also delays the proper management of the patients.[1]

On routine hematoxylin and eosin (H & E) staining, many tumors can look alike in their morphology. So much so, that even with expert eyes, it becomes nearly impossible to give a definite diagnosis in all cases. One such scenario is small round blue cell tumors. These are neoplasms that are highly undifferentiated and contain similar-looking growth of small round cells. Individual cells depict increased basophilic staining and a high N/C ratio.[2] They are further characterized by sheets of primitive-looking cells lacking a clue of the cell of origin at routine H & E.[3] Following tumors are included in the differential diagnosis (d/d) of malignant small round blue cell tumors (MSRBCT). Non-Hodgkin lymphoma, Retinoblastoma, Lymphoblastic Lymphoma, Hepatoblastoma, Ewing Sarcoma/Primitive Neuroectodermal Tumor, Neuroblastoma, Synovial Sarcoma, Wilm's tumor, Neuroendocrine carcinoma, Osteosarcoma, Desmoplastic Small Round Cell Tumor, Nasopharyngeal Carcinoma, Dysgerminoma, Mesenchymal Chondrosarcoma, Dendritic cell Tumor, Malignant Melanoma (Small Cell variant) and Rhabdomyosarcoma.[2,4,5] This list can be more gigantic if we consider site-related blue cell tumors.[6–8] These tumors are more usually diagnosed in the pediatric age group, nevertheless, they are seen in adults also.[9,10] Accurate sub-categorization and the final diagnosis are highly important because the treatment of each tumor can be unique. For example, chemotherapy is the mainstay of treatment in sarcomas and lymphoma. Anti-CD20 therapy is the mainstay option in CD20-positive lymphoma. Melanomas are treated by surgery and interferon therapy. Chemotherapy is the treatment of choice in Extra-gonadal germ cell tumors and neuroendocrine tumors. Moreover, Desmoplastic Small Round Cell Tumors are treated with a totally different approach.[4,11–13] Therefore, many diagnostic modalities are in practice for precise diagnosis of this entity, including Immunohistochemistry (IHC)[10], electron microscopy, FISH (Fluorescence in situ hybridization)[3], cytogenetic studies and molecular techniques[14] like reverse transcriptase polymerase chain reaction.[5,15] Cytogenetic techniques are best for confirmatory diagnosis but they are too costly for poor folks.[16] So immunohistochemistry remains the mainstay in these setups.

Pakistan is also a resource-poor country, where access to sophisticated diagnostic modalities is limited to a few setups, which are inaccessible to the common public. Faisalabad is the third largest city in Pakistan, but it sophisticated laboratory setups, and most of the malignant challenging cases are sent to referral labs located in Lahore or Karachi. Our rationale is to use immunohistochemistry at the local level to sub-categorize this diverse group as a minimum requirement and thereby facilitate prompt management and treatment.

Objective:

The objective of the study is to sub-categorize pediatric Small Round Blue Cell Tumors using immunohistochemistry.

Materials and Methods

Study design: Descriptive cross-sectional study.

Setting: Meezan private lab, Faisalabad, Pakistan.

Duration of study: 4 years, from January 2017 to December 2020.

Sample size: 46 cases of Pediatric Malignant Small Round Blue Cell Tumor.

Sampling technique: Non-probability purposive sampling

Inclusion criteria:

- All cases up to age 15, are diagnosed as Malignant Small Round Blue Cell Tumor on routine hematoxylin and eosin staining.

Exclusion criteria:

- Autolyzed tissue
- Immuno-histochemistry could not be done, because of technical or logistics issues.

Data collection procedure

After ethical approval, 46 cases, which fulfilled the inclusion and exclusion criteria were selected for the study. All these cases were subjected to immunohistochemistry. The IHC technique used was based on the Peroxidase anti-peroxidase (PAP) method. The protocol used is as follows:

1. Cut tissue sections 2.0 – 4.0 microns thick and spread wrinkle-free on the slide.
2. Put the slides on a Hot plate 60°-65° for 45 to 50 min.
3. For deparaffinisation, gave 3-changes of Xylene 5 min each.

4. Rehydrated the tissue with graded isopropanol (100%, 80%, 70%, 50%) 5 min each.
5. Put distilled water for 3 to 5 min two changes.
6. Then put in antigen retrieval solution (target retrieval solution) in Kortil Coplin Jar: Dilution 1:50. pH was 9.0 for CD5, CD10, CD3, CD30, CD99 and 2.5 pH for Myogenin, Ki-67, WT1. All remaining had pH 6.
7. Put in the water bath at 99.5° for 45 mins - 1 hour.
8. Took out from water bath and put at room temperature.
9. Then washed in wash buffer solution for 10 min, two changes. Dilution 1:20 (pH 7.6)
10. Poured peroxidase blocking reagent on slide covering the tissue area and put in Humidity chamber for 10 min.
11. Washed again in wash buffer for 10 min, two changes.
12. Poured 50 ul of primary antibody on tissue area and put in Humidity chamber for 45 min to 1 hour (as per literature).
13. Again washed in wash buffer for 10 min, two changes.
14. Poured 50 ul of the secondary antibody (HRP) on the tissue area and put in a Humidity chamber for 45 min to 1 hour.
15. Washed again in wash buffer for 10 min, two changes.
16. Added Dabe chromogin 50 ul on tissue area for 3-5 min (Dabe chromogin 50 ul and substrate 1 ml).
17. Washed in distilled water for 3 to 5 min.
18. Counter-stained with hematoxylin by 3-5 dips.
19. Washed in Tap water for 3 to 5 min.
20. Put the slides rack in Proponol for 3 cycles of 5 min, 3 min, and 5 min respectively.
21. Air dried and given 3 cycles of Xylene 5 min, 3 min, and 5 min respectively.
22. Mounting with DPX (DisrteneDibutyl-Pthalate Xylene) and then observed the slide.

Based on site and morphological clues, initially Leukocyte common antigen (LCA), Myogenin, Cytokeratin (CK), Desmin, chromogranin, Neuron-specific enolase (NSE), S-100, Smooth muscle actine (SMA) and CD99 were used. Further immune stains panels were used afterward, as and when needed like CD20, CD3, CD30, BCL2, CD117, Ki-67, Tdt, synaptophysin, SMA, CD56, Melan A, HMB45, and WT1. The results were analyzed independently by 2 histopathologists. Staining intensity was graded as negative, or weak, moderate to strong positive. The extent of positive IHC reaction was scored as focal (< 10%), patchy (10-50%), or diffuse (>50%)[17], and the final diagnosis was rendered.

Data analysis:

All the collected information was entered and analyzed using SPSS version 24. The qualitative variables like gender, site, and diagnosis were presented by calculating frequency and percentage.

Results

Out of 46 cases of MSRBCT, Rhabdomyosarcoma was the highest in frequency i.e. 8 (17.4%), followed by Ewing's sarcoma/PNET 7 (15.2%). Both Diffuse large B cell lymphoma and Neuroblastoma were 5 each in number (10.9%). Non-Hodgkins lymphoma as a whole, was 13 (28.2%), including 5 cases (10.9%) of diffuse large B cell lymphoma, 4 cases (8.7%) of T lymphoblastic lymphoma, 3 cases (6.5%) of Burkitt's lymphoma, and only 1 case (2.2%) of NK/T cell lymphoma. (Table 1)

Table 1: Frequency of subcategories of Malignant Small Round Blue Cell Tumors

Tumors	*Frequency*	*Percent*
Diffuse Large B Cell Lymphoma	5	10.9
T Lymphoblatic lymphoma	4	8.7
Burkitt's lymphoma	3	6.5
Nk/T cell lymphoma	1	2.2
Nasopharyngeal carcinoma	1	2.2
Ewing's sarcoma/PNET	7	15.2
Rhabdomyosarcoma	8	17.4
Synovial sarcoma	4	8.7
Malignant Melanoma	1	2.2
Neuroblastoma	5	10.9
Germ cell tumor	3	6.5
Rhabdoid tumor	1	2.2
Desmoplatic Small Round cell tumor	1	2.2
Round blue cell tumor, Unclassified	2	4.3
Total	46	100.0

Common pediatric age group for MSRBCT is below 5 years of age i.e. 22 (47.9%) and least commonly from 13 to 15 years of age i.e. 10 (21.7%). (Table 2)

Table 2: Frequency of different categories of Malignant Small Round Blue Cell Tumors (MSRCT) in different age groups

Differential diagnosis of Round blue cell tumor	*Age groups*			*Total*
	1 month to 5 years	>5 to 12 years	> 12 to 15 years	
Diffuse Large B Cell Lymphoma	1	4	0	5
T Lymphoblatic lymphoma	1	1	2	4
Burkitt's lymphoma	3	0	0	3
Nk/T cell lymphoma	0	1	0	1
Nasopharyngeal carcinoma	0	0	1	1
Ewing's sarcoma/PNET	2	3	2	7
Rhabdomyosarcoma	6	1	1	8
Synovial sarcoma	0	1	3	4
Malignant Melanoma	0	1	0	1
Neuroblastoma	4	0	1	5
Germ cell tumor	2	1	0	3
Rhabdoid tumor	1	0	0	1
Desmoplatic Small Round cell tumor	1	0	0	1
Round blue cell tumor, Unclassified	1	1	0	2
Total	**22 (47.9%)**	**14 (30.4%)**	**10 (21.7%)**	**46 (100%)**

If we consider the site of origin, then 24% of cases were biopsied from the cervical lymph node, followed by abdominal mass (11%) and Retroperitoneum (9%). (Table 3)

Table 3: Frequency of Individual categories and their site of origin

Tumors	*Frequency*	*Site of Origin*
Diffuse Large B Cell Lymphoma	5	Cervical lymph node (1), Intestine (1), abdominal mass (2), Retroperitoneum (1),
T Lymphoblatic lymphoma	4	Cervical lymph node (2), Nasopharynx (2),
Burkitt's lymphoma	3	Liver mass (1), Nasopharynx (1), Intestine (1)
Nk/T cell lymphoma	1	Abdominal mass (1)
Nasopharyngeal carcinoma	1	Cervical lymph node (1)
Ewing's sarcoma/PNET	7	Cervical lymph node (2), Bone (1), Chest mass (2), Pelvic mass (1), wrist joint (1)
Rhabdomyosarcoma	8	Cervical lymph node (3), Inguinal lymph node (1), Liver mass (1), Testis (1), Urinary bladder mass (1), Eyelid (1)
Synovial sarcoma	4	Pelvic mass (1), Thigh mass (3)
Malignant Melanoma	1	Scalp mass (1)
Neuroblastoma	5	Cervical lymph node (2), Retroperitoneum (3),
Germ cell tumor	3	Testis (1), Abdominal mass (1), Ovarian mass (1)
Rhabdoid tumor	1	Kidney mass (1)
Desmoplatic Small Round cell tumor	1	Abdominal mass (1)
Round blue cell tumor, Unclassified	2	Left ankle (1), Pelvic mass (1)
Total	**46**	

It was noticed in the present study, that some tumors were only seen in males like DLBCL, Burkitt's, and Desmoplastic small round cell tumors, while some were only seen in females like NK/T cell lymphoma and malignant melanoma. Higher percentages of Rhabdomyosarcoma, Ewings, and Neuroblastoma were present in males and germ cell tumors were frequent in females. (Figure 1)

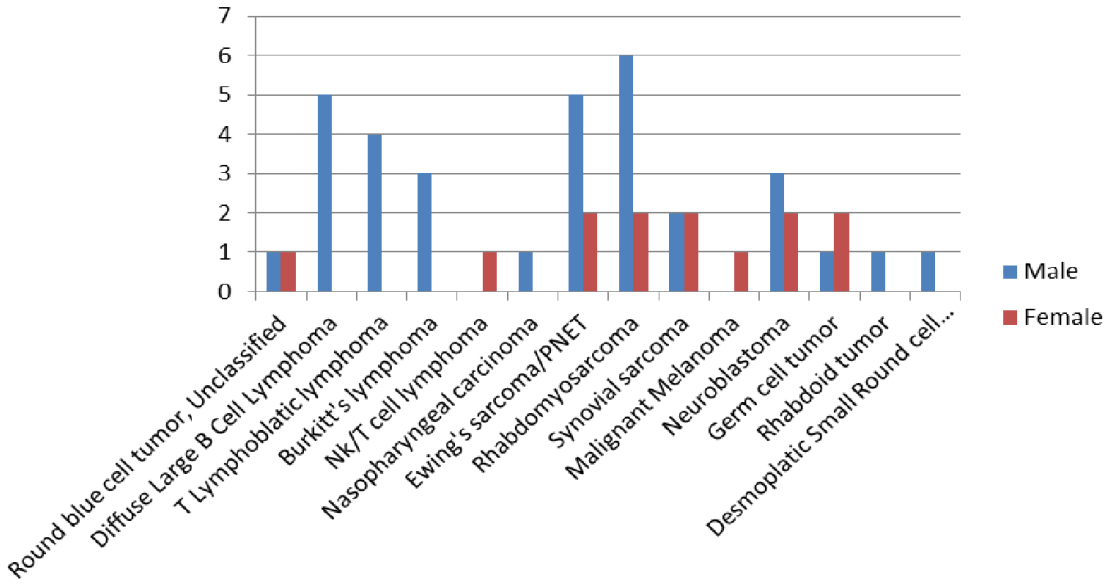

Figure 1: Gender distribution among Malignant Small Round Blue Cell Tumors

In our study, one case from the ankle and 2nd case from the pelvic area remained undiagnosed with available panels of immunostains

Discussion

Numerous studies have mentioned the benefits of IHC for confirmatory diagnosis of pediatric small round blue cell tumors.[2,5,17–23] Moreover, Thomas et al. reported that "Immunohistochemistry changed the diagnosis of 24% of the cases".[9] In our article, We used established panels of antibodies that have been used by other studies in the literature.[2,4,10,16,21] However, some research papers, used novel antibodies like PAX7[19], NKX2.2[17], BCOR[24], ETV4[25,] etc for differential diagnosis, claiming that they are better and specific. Nevertheless, financial limitations did not allow us to use these novel antibodies.

In the current study, Lymphomas were most frequent, when they were grouped together i.e. 13(28.2%) out of 46 cases. It included 5 cases (10.9%) of diffuse large B cell lymphoma, 4 cases (8.7%) of T lymphoblastic lymphoma, 3 cases (6.5%) of Burkitt's lymphoma, and 1 case (2.2%) T/NK cell lymphoma (Refer to Table 1). This fact was also reported by Thomas et al[9] and Patel et al.[10] Nevertheless, the exact frequency was different in all articles. This might be due to racial and geographical differences. Contrary to it, Round Cell Tumors of the sinonasal location showed more carcinomas than lymphomas[5], but this is a location-based difference since other studies also highlighted this particular aspect.[6,26] Based on this fact, it can be narrated that IHC panels for MSRBCT, should include lymphoma panels like LCA (CD45), CD30, CD20, CD3, CD5, Tdt, Cyclin D1, and Ki-67, etc. Other specific markers like CD10, BCL2, and ALK can be added to the above panel, if and when needed.

If we consider individual tumors, then Rhabdomyosarcoma is the highest tumor in our study 8/46(17.4%). Out of a total of 21 soft tissue tumors in the current study, 8 are Rhabdomyosarcoma with a percentage of 38%. It is consistent with other studies, which mentioned that it has an incidence of 40 % and is the most frequent pediatric soft tissue malignancy.[2,27] Site of biopsy for Rhabdomyosarcoma was variable i.e. cervical region[3], and 1 case each from the inguinal region, urinary bladder, liver, testis, and eyelid. And this particular aspect has been reported by other studies also.[2,27–29] Ewing's/PNET is 3rd in the list comprising of 7 cases (15.2%), biopsied mostly from the cervical area, followed by the extra-skeletal site of the chest. Thigh and shoulder, as the extra-skeletal site was also mentioned by Patel et al.[4] Shi Wei et al. reported that "Ewing's/PNET is 2nd most common bone malignancy in children and young adults with 6-8% incidence and 20% could be extra skeletal".[3] The reason for putting Ewing's and PNET in one group is

that they depict the same morphology and cytogenetic alteration. It has been noticed that although Ewing's sarcoma of bone is mostly undifferentiated, PNET shows some degree of neuro-ectodermal differentiation.[30] Synovial sarcoma is 4(8.7%) in number and biopsy is taken from pelvic mass[1] and thigh mass.[3] Many other studies also mentioned the site of origin of synovial sarcoma, near the joints like the thigh and knee.[2,17,31] Neuroblastoma is 5 out of 46 in the current study with 10.9%. Sharma et al reported it as the "3rd most malignant extracranial solid tumor of childhood, arising from primitive neural crest cells".[2] In our research, 3/5 of Neuroblastoma cases were taken from retroperitoneum, which is also highlighted by Machado I et.al.[17] In our study, there was only 1 (2.2%) case of desmoplastic small round cell tumor (DSRCT), arising from the abdominal tissue. Bulbul et al. also exclaimed that "DSRCT has a predilection for abdominal and pelvic cavity".[13]

It was noticed that some tumors were only seen in males like DLBCL, Burkitt's, and Desmoplastic Small Round Cell Tumor, while some were only seen in females like NK/T cell lymphoma and malignant melanoma. Higher percentages of Rhabdomyosarcoma, Ewings, and Neuroblastoma were present in males and germ cell tumors were common in females (Figure 1). Likewise, Thompson et al and Shi Wei et.al. quoted slight male predominance of Ewing's/PNET and Neuroblastoma.[3,5] However, the less number of cases limits the ability to clearly define gender differences in the current study. Regarding age group, since these MSRBCTs are more commonly seen in children and younger age groups, so we also found that 22 (47.9%) patients were within 5 years of age at the time of diagnosis. This particular aspect has been shown by many studies.[9,16] Two cases (4.3%), ankle and pelvic area respectively, could not be classified even after the use of all available antibodies. This aspect concurs with Patel et al[10], in which the percentage of unclassified cases was 3.75%. The cause in our study was unequivocal IHC results and the unavailability of novel antibodies. To solve this matter, wider IHC panels and sophisticated techniques like EM, cytogenetic and molecular techniques should be used for further categorization.

Limitation of Study

Limited resources and a lack of cytogenetics studies hindered the proper sub-categorization of some of our cases. Moreover, newer IHC antibodies, which are more promising and specific, as reported in a few studies were not used because of economic issues. We suggest future research should be performed, to solve above stated deficiencies.

Conclusion

For true and accurate sub-categorization of Pediatric small round blue cell tumors, Immunohistochemistry is an essential diagnostic tool, which should be used in all cases.

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References

1. Gazula S, Rani Vl, Jonathan G, Kumar Nn. Extraskeletal Ewing's sarcoma masquerading as infantile benign neck mass. J Indian Assoc Pediatr Surg. 2019;24(3):209.
2. Sharma S, Kamala R, Nair D, Ragavendra Tr, Mhatre S, Sabharwal R, et al. Round cell tumors: Classification and immunohistochemistry. Indian J Med Paediatr Oncol [Internet]. 2017 [cited 2019 Jan 14];38(3):349. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5686981/
3. Wei S, Siegal GP. Round Cell Tumors of Bone : An Update on. Adv Anat Pathol [Internet]. 2014 [cited 2019 Jan 14];21(5):359–72. Available from: https://journals.lww.com/anatomicpathology/Abstract/2014/09000/Round_Cell_Tumors_of_Bone__An_Update_on_Recent.5.aspx
4. Patel A, Patel M, Bhagat V, Naik K. Role of immunohistochemistry in the differential diagnosis of malignant small round cell tumor: a study of 38 cases. Int J Res Med Sci [Internet]. 2015 [cited 2019 Jun 17];3(12):3833–9. Available from: https://pdfs.semanticscholar.org/8791/92784eb4f7236ee61e6f685807ebf5dab9f8.pdf
5. Thompson LDR. Small round blue cell tumors of the sinonasal tract: A differential diagnosis approach. Mod Pathol [Internet]. 2017 [cited 2019 Jun 17];30(s1):S1–26. Available from: https://www.nature.com/articles/modpathol2016119
6. Rooper LM, Bishop JA. Sinonasal Small Round Blue Cell Tumors. Surg Pathol Clin [Internet]. 2016 [cited 2019 Jan 14];10(1):103–23. Available from: https://www.sciencedirect.com/science/article/pii/S1875918116300927
7. Narayanan G, Rajan V, Preethi TR. Primitive neuroectodermal tumors of the kidney. Baylor Univ Med Cent Proc [Internet]. 2017 Apr 11 [cited 2019 Jun 17];30(2):205–8. Available from: https://www.tandfonline.com/doi/full/10.1080/08998280.2017.11929588
8. Tsukamoto Y, Futani H, Yoshiya S, Watanabe T, Kihara T, Matsuo S, et al. Primary undifferentiated small round cell sarcoma of the deep abdominal wall with a novel variant of t(10;19) CIC-DUX4 gene fusion. Pathol Res Pract [Internet]. 2017 [cited 2019 Jun 17];213(10):1315–21. Available from: https://www.sciencedirect.com/science/article/pii/S0344033817302558
9. Thomas J, AA O-E. Immunocytochemistry in the diagnosis of small blue cell tumours of childhood. West Afr J Med [Internet]. 2006 [cited

2019 Jun 17];25(3):199–204. Available from: https://www.ajol.info/index.php/wajm/article/view/28278
10. Patel MM, Dhandha ZB, Italiya SL, Shah MB, Kaptan KR, Mansuri BM, et al. Role of Immunohistochemistry in Differential Diagnosis of Round Cell Tumor. Indian J Res. 2013;3(5):217–20.
11. Frezza AM, Benson C, Judson IR, Litiere S, Marreaud S, Sleijfer S, et al. Pazopanib in advanced desmoplastic small round cell tumours: a multi-institutional experience. Clin Sarcoma Res [Internet]. 2014 Dec 29 [cited 2019 Jan 14];4(1):7. Available from: http://clinicalsarcomaresearch.biomedcentral.com/articles/10.1186/2045-3329-4-7
12. Menegaz BA, Cuglievan B, Benson J, Camacho P, Lamhamedi-Cherradi S, Leung CH, et al. Clinical Activity of Pazopanib in Patients with Advanced Desmoplastic Small Round Cell Tumor. Oncologist [Internet]. 2018 [cited 2019 Jun 17];23(3):360–6. Available from: http://theoncologist.alphamedpress.org/content/23/3/360.short
13. Bulbul A, Fahy BN, Xiu J, Rashad S, Mustafa A, Husain H, et al. Desmoplastic Small Round Blue Cell Tumor: A Review of Treatment and Potential Therapeutic Genomic Alterations. Sarcoma [Internet]. 2017 [cited 2019 Jun 17];2017:1–12. Available from: https://www.hindawi.com/journals/sarcoma/2017/1278268/abs/
14. Koelsche C, Hartmann W, Schrimpf D, Stichel D, Jabar S, Ranft A, et al. Array-based DNA-methylation profiling in sarcomas with small blue round cell histology provides valuable diagnostic information. Mod Pathol [Internet]. 2018 [cited 2019 Jun 17];31(8):1246–56. Available from: https://europepmc.org/abstract/med/29572501
15. Owosho AA, Estilo CL, Huryn JM, Zhang L, Fletcher CDM, Antonescu CR. Head and Neck Round Cell Sarcomas: A Comparative Clinicopathologic Analysis of 2 Molecular Subsets: Ewing and CIC-Rearranged Sarcomas. Head Neck Pathol [Internet]. 2017 Dec 23 [cited 2019 Jun 17];11(4):450–9. Available from: http://link.springer.com/10.1007/s12105-017-0808-z
16. Qiaoxin Li, Wenli Cui, Gulinaer Abulajiang, Yuqing Ma, Xia Liu, Wei Zhang, et al. Application of Immunohistochemistry in the Diagnosis of Small Round Blue-Cell Tumors of Soft Tissue. Clin Lab [Internet]. 2013 [cited 2019 Jan 14];59(01+02/2013). Available from: https://www.clin-lab-publications.com/article/1564
17. Machado I, Yoshida A, Morales MGN, Abrahão-Machado LF, Navarro S, Cruz J, et al. Review with novel markers facilitates precise categorization of 41 cases of diagnostically challenging, "undifferentiated small round cell tumors". A clinicopathologic, immunophenotypic and molecular analysis. Ann Diagn Pathol [Internet]. 2018 Jun [cited 2019 Jun 18];34:1–12. Available from: http://www.ncbi.nlm.nih.gov/pubmed/29661713
18. Magro G, Brancato F, Musumeci G, Alaggio R, Parenti R, Salvatorelli L. Cyclin D1 is a useful marker for soft tissue Ewing's sarcoma/peripheral Primitive Neuroectodermal Tumor in children and adolescents: A comparative immunohistochemical study with rhabdomyosarcoma. Acta Histochem [Internet]. 2015 [cited 2019 Jan 14];117(4–5):460–7. Available from: https://www.sciencedirect.com/science/article/pii/S0065128115000343
19. Charville GW, Varma S, Forgó E, Dumont SN, Zambrano E, Trent JC, et al. PAX7 expression in rhabdomyosarcoma, related soft tissue tumors, and small round blue cell neoplasms. Am J Surg Pathol [Internet]. 2016 [cited 2019 Jan 14];40(10):1305–15. Available from: https://www.ingentaconnect.com/content/wk/ajsp/2016/00000040/00000010/art00002
20. McCuiston A, Bishop JA. Usefulness of NKX2.2 Immunohistochemistry for Distinguishing Ewing Sarcoma from Other Sinonasal Small Round Blue Cell Tumors. Head Neck Pathol [Internet]. 2018 Mar 14 [cited 2019 Jun 17];12(1):89–94. Available from: http://link.springer.com/10.1007/s12105-017-0830-1
21. Yamada Y, Kuda M, Kohashi K, Yamamoto H, Takemoto J, Ishii T, et al. Histological and immunohistochemical characteristics of undifferentiated small round cell sarcomas associated with CIC-DUX4 and BCOR-CCNB3 fusion genes. Virchows Arch [Internet]. 2017 Apr 14 [cited 2019 Jun 17];470(4):373–80. Available from: https://link.springer.com/article/10.1007/s00428-017-2072-8
22. Hung YP, Lee JP, Bellizzi AM, Hornick JL. PHOX2B reliably distinguishes neuroblastoma among small round blue cell tumours. Histopathology [Internet]. 2017 Nov [cited 2019 Jun 17];71(5):786–94. Available from: http://doi.wiley.com/10.1111/his.13288
23. Magro G, Salvatorelli L, Alaggio R, D'Agata V, Nicoletti F, Di Cataldo A, et al. Diagnostic utility of cyclin D1 in the diagnosis of small round blue cell tumors in children and adolescents. Hum Pathol [Internet]. 2017 [cited 2019 Jan 14];60:58–65. Available from: https://www.sciencedirect.com/science/article/pii/S0046817716302647
24. Kao YC, Sung YS, Zhang L, Jungbluth AA, Huang SC, Argani P, et al. BCOR overexpression is a highly sensitive marker in round cell sarcomas with BCOR genetic abnormalities. Am J Surg Pathol [Internet]. 2016 [cited 2019 Jan 14];40(12):1670–8. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5106294/
25. Le Guellec S, Velasco V, Pérot G, Watson S, Tirode F, Coindre JM. ETV4 is a useful marker for the diagnosis of CIC-rearranged undifferentiated round-cell sarcomas: A study of 127 cases including mimicking lesions. Mod Pathol [Internet]. 2016 [cited 2019 Jan 14];29(12):1523–31. Available from: https://www.nature.com/articles/modpathol2016155
26. Thompson LDR, Franchi A. New tumor entities in the 4th edition of the World Health Organization classification of head and neck tumors: Nasal cavity, paranasal sinuses and skull base. Virchows Arch [Internet]. 2018 Mar 25 [cited 2019 Jun 17];472(3):315–30. Available from: https://link.springer.com/article/10.1007/s00428-017-2116-0
27. Hibbitts E, Chi Y, Hawkins DS, Barr FG, Bradley JA, Dasgupta R, et al. Refinement of risk stratification for childhood rhabdomyosarcoma using FOXO1 fusion status in addition to established clinical outcome predictors: A report from the Children's Oncology Group. Cancer Med [Internet]. 2019 Oct 27 [cited 2020 Apr 21];8(14):6437–48. Available from: https://onlinelibrary.wiley.com/doi/abs/10.1002/cam4.2504
28. Córdoba Rovira SM, Inarejos Clemente EJ. Rabdomiosarcoma infantil. Radiologia [Internet]. 2016 Nov 1 [cited 2020 Apr 21];58(6):481–90. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0033833816301400
29. Bhaskar Bhuvan L, Radhakrishnan V, Raja A, Ganesarajah S, Sagar T. Outcomes in rhabdomyosarcoma: Experience from a tertiary cancer center in India. Cancer Res Stat Treat. 2019;2(1):4.
30. Machado I, Navarro L, Pellin A, Navarro S, Agaimy A, Tardío JC, et al. Defining Ewing and Ewing-like small round cell tumors (SRCT): The need for molecular techniques in their categorization and differential diagnosis. A study of 200 cases. Ann Diagn Pathol [Internet]. 2016 [cited 2019 Jan 14];22:25–32. Available from: https://www.sciencedirect.com/science/article/pii/S1092913416300296
31. Scheer M, Blank B, Bauer S, Vokuhl C, Stegmaier S, Feuchtgruber S, et al. Synovial sarcoma disease characteristics and primary tumor sites differ between patient age groups: a report of the Cooperative Weichteilsarkom Studiengruppe (CWS). J Cancer Res Clin Oncol. 2020 Apr 1;146(4):953–60.