

Immunohistochemical detection of FLI-1 protein expression in Ewing Sarcoma/ peripheral primitive neuroectodermal tumour: A study of 50 cases

Safina Ahmed,¹ Shoaib Naiyar Hashmi,² Hafeez-ud-Din³

Abstract

Objective: To determine friend leukaemia integration 1 transcription factor protein expression in cases of Ewing sarcoma.

Methods: This retrospective, descriptive study was conducted at the Armed Forces Institute of Pathology, Rawalpindi, Pakistan, and comprised data of diagnosed cases of Ewing sarcoma related to the period from February 2013 to December 2014. Clinico-pathological features, including patient age, gender and site of biopsy were studied. Positivity of immunohistochemical markers such as cluster of differentiation 99(membranous staining) and Friend leukaemia integration 1 transcription factor (nuclear staining) were noted. SPSS17 was used for data analysis.

Results: Of the 50 Ewing sarcoma cases, 26(52%) related to women and 24(48%) to men. The overall mean age was 17+11.53 years (range: 3 to 42 years). Moreover, 30(60%) patients had presented with bone swelling or growth whereas 20(40%) had presented with soft tissue swelling. The site of presentation was upper extremities in 16(32%) patients, lower extremities in 14(28%), maxilla in 7(14%), chest wall in 6(12%), paraspinal region in 4(8%), scalp in 2(4%) and retroperitoneum in 1(2%).

Membranous positivity for cluster of differentiation 99 was seen in 48(98%) cases. Nuclear positivity for Friend leukaemia integration 1 transcription factor was seen in 39(78%) cases.

Conclusions: Friend leukaemia integration 1 transcription factor was found to be a useful marker in diagnosing Ewing sarcoma/peripheral primitive neuroectodermal tumour. However, its positivity was more dependable when it was used in combination with other markers such as cluster of differentiation 99.

Keywords: Ewing sarcoma, Immunohistochemistry, CD-99, FLI-1. (JPMA 66: 1296; 2016)

Introduction

Ewing sarcoma/peripheral primitive neuroectodermal tumour (EWS/PNET) is a malignant small round blue cell tumour. It is the second most common bone tumour in children and adolescents. The majority of 'EWS (around 85%) occur in the bones; however they can occur in the soft tissues as well.¹

A variety of tumours have overlapping morphological features such as EWS, rhabdomyosarcoma, lymphoma, desmoplastic small round cell tumour, etc. These tumours are categorised as small round blue cell tumours. The diagnosis of EWS is difficult because of its broad differential diagnosis. Although the cytogenetic detection of tumour type specific translocation is a gold standard for diagnosis, such techniques are not widely available.² Immunohistochemistry(IHC) is one of the most prevalent and convenient methods for making a correct diagnosis.³

Microscopically, classical EWS is composed of sheets of

monotonous round to oval cells with round primitive-appearing nuclei and moderate amount of clear to amphophilic cytoplasm. The nuclei have smooth contours with single inconspicuous nucleolus and finely dispersed chromatin. The lesions are markedly cellular with little intervening stroma.⁴

IHC studies are primarily carried out to diagnose EWS and to exclude other small round cell tumours, especially lymphoma and rhabdomyosarcoma. Small-cell osteosarcoma and mesenchymalchondrosarcoma are also considered in the differential diagnosis. Synovial sarcoma may also exhibit round cell morphology similar to EWS. Various IHC markers are expressed by EWS/PNET family of tumours, such as neuron-specific enolase (NSE), cluster of differentiation (CD) 57, Synaptophysin and CD99. All these markers are expressed by other tumours as well. However, lymphoblastic lymphoma would express the lymphoid markers, i.e., CD45, CD3, CD20, and terminal deoxynucleotidyl transferase (TdT) which will help in its differentiation. Rhabdomyosarcoma would be positive for skeletal muscle markers e.gdesmin, myogenin, myo-D1, and myoglobin. Similarly synovial sarcoma would express pancytokeratins, epithelial

¹Department of Pathology, ^{2,3}Consultant Histopathologist, Armed Forces Institute of Pathology, Rawalpindi.

Correspondence: Safina Ahmed. Email: a_safina@yahoo.com

membrane antigen (EMA) and BCL2 (B-cell lymphoma-2).⁵ Initially, CD99 was considered to be a specific EWS marker. CD99 is a membrane-associated protein also known as MIC-2 (Single-chain type-1 glycoprotein). It plays a major role in cell adhesion, migration and apoptosis. Later on, it was found that other tumours, including lymphoblastic lymphoma, mesenchymalchondrosarcoma, and synovial sarcoma also expressed CD99. A membranous pattern of CD99 staining is believed to be more specific for EWS rather than cytoplasmic staining, but it is now known that this too can be found in other tumours, for example, synovial sarcomas.⁶

Approximately 90% of EWS/PNET have a specific translocation t(11; 22), which results in a chimeric fusion EWS-Friend leukaemia integration 1 transcription factor (FLI-1) protein. The reciprocal translocation t(11; 22) results in juxtaposition of the amino terminal domain of EWS to the carboxyl-terminus of FLI-1. FLI-1 is normally expressed in endothelial cells and haematopoietic cells. It is a member of the erythroblastosis virus-associated transforming sequences (ETS) family of deoxyribonucleic acid (DNA)-binding transcription factors, and it is involved in cellular proliferation and tumourigenesis. Other translocations are also detected in EWS such as t(21; 22) resulting in chimeric fusion EWS-ETS-related gene (ERG) protein. IHC for FLI-1 (nuclear positivity) is sensitive and highly specific for the diagnosis of EWS/PNET.⁷

The EWS-FLI gene fusions found in 'EWS provide opportunities for new approaches to treatment. As the genetic changes responsible for transformation, growth and metastases of EWS are becoming clearer, potentials for new therapeutic targets are increasing. This has given us an opportunity to discover predictive biomarkers for the patients which will help them in getting benefit from targeted therapy.⁸

The present study was planned to determine the efficacy of FLI-1 protein expression in diagnosing EWS along with other conventional IHC markers.

Materials and Methods

This retrospective, descriptive study was conducted at the Armed Forces Institute of Pathology (AFIP), Rawalpindi, Pakistan, and comprised data of diagnosed EWS cases retrieved from archives related to period from February 2013 to December 2014. Clinico-pathological features, including patient age, gender and site of biopsy were studied. Positivity of IHC markers such as CD-99 and FLI-1 were noted. Cases in which FLI-1 stain was not available were included and FLI-1 stain was applied. IHC was done manually. IHC techniques detect antigens in tissue sections by means of immunological and chemical

reactions. This technique is highly sensitive and specific and can detect a wide variety of antigens.⁹ For staining, the slides were deparaffinised and rehydrated. After antigen retrieval IHC stain was applied. Again, the slides were dehydrated and stabilised with mounting medium.

Membranous staining of CD99 and nuclear positivity of FLI-1 stain were identified to label positive and negative cases. Data was analysed using SPSS-17. Descriptive statistics was used for quantitative variables while frequency and percentage were calculated for qualitative variables.

Results

Of the 50 EWS cases, 26(52%) related to women and 24(48%) to men. The overall mean age was 17±11.53 years (range: 3 to 42 years).

Besides, 30(60%) patients had presented with bone swelling or growth whereas 20(40%) had presented with soft tissue swelling. The site of presentation was upper extremities in 16(32%) patients, lower extremities in 14(28%), maxilla in 7(14%), chest wall in 6(12%), paraspinal region in 4(8%), scalp in 2(4%) and retroperitoneum in 1(2%).

Membranous positivity for CD99 was seen in 48(98%) cases while nuclear positivity for FLI-1 was seen in 39(78%) cases.

Discussion

EWS/PNET is the second most common bone tumour among children and young adults.¹ EWS has a wide spectrum of differential diagnosis, and is morphologically indistinguishable from other round blue cell tumours. Therefore, IHC markers are always required to confirm the diagnosis.²

In the present study the age of the patients ranged from 3 years to 42 years whereas the mean age was 17 years. Amongst them 26(52%) were females and 24(48%) were males. A 2003 study in Saudi Arabia showed that skeletal EWS had predilection for second decade of life whereas extra skeletal EWS predominantly occurred in the first decade of life.¹⁰ A study on 14 patients showed comparable clinical features. The age range in that study was 12 years to 77 years and the mean age was 17 years. However, women outnumbered men in that study.¹¹

The most common site of presentation in the current study was upper extremity. In another study, seven tumours occurred in the extremities, five in the trunk wall and two in the head region.¹¹

In this study, membranous positivity for CD99 was seen in

48(98%) cases. Nuclear positivity for FLI-1 was seen in 39(78%) cases. A study showed strong positivity for CD99 exhibiting membranous staining in all cases. However, instead of using IHC stain for FLI-1, that study used fluorescence in situ hybridisation (FISH) and reverse transcriptase-dependent polymerase chain reaction (RT-PCR) analysis for EWS-FLI1 fusion gene. The FISH analysis in that study revealed positive gene fusion in 10/14 cases.¹¹

Another study on four EWS cases involving vulva and vagina revealed that CD99 was positive in all cases whereas FLI-1 was positive in 50% cases. All cases were diffusely positive for vimentin, but negative for desmin.¹²

A study conducted as cluster analysis of EWS, synovial sarcoma and malignant peripheral nerve sheath tumour showed CD99 positivity in 100% cases of EWS. On the contrary, FLI-1 was positive in 65% cases only. The authors in that study suggested that a membranous pattern of CD99 staining was more specific than cytoplasmic staining, but this too can be found in other tumours e.g. synovial sarcoma. Similarly, FLI-1 positivity, initially reported to be useful in EWS was also found to stain other tumours such as lymphoblastic lymphoma, synovial sarcoma and malignant peripheral nerve sheath tumours, each with strong nuclear staining. The study suggested that FLI-1 was most useful when used in combination with CD99.¹³

Another study done on 132 cases of small round blue cell tumours revealed FLI-1 expression in 29(71%) EWS/pPNET cases. Some of the lymphoblastic lymphomas and a case of desmoplastic small round cell tumour also showed FLI-1 positivity. The study suggested that although the EWS/FLI-1 fusion gene is specific for EWS, FLI-1 protein expression is not. So IHC detection of FLI-1 is valuable in conjunction with CD99 positivity. Moreover, FLI-1 protein expression is also helpful in distinguishing EWS from other tumours that may be CD99-positive.³

The crux of discussion is that FLI-1 positivity in EWS is helpful in the diagnosis when it is used in conjunction with other markers such as CD99. It is not surprising that some cases of EWS are FLI-1 negative as all tumours do not carry the classic t(11; 22) translocation.

Conclusion

FLI-1 was found to be a useful marker in diagnosing EWS/PNET. However, its positivity was more dependable when used in conjunction with other markers such as CD99.

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Conflict of Interest: No.

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