SERVIZIO SANITARIO REGIONALE
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Istituto Ortopedico Rizzoli di Bologna
Istituto di Ricovero e Cura a Carattere Scientifico

"Angiomatoid Fibrous Histiocytoma: a single institution case-series"

Study code	**AFH**
Sponsor's Name and Address:	Istituto Ortopedico Rizzoli Via di Barbiano 1/10 40136 Bologna Italy
Study Number/Version/Date:	Vers 1.0 03 Sep 2018
Coordinating Center:	IRCCS Istituto Ortopedico Rizzoli Pathology Unit Via Pupilli 1 40136 Bologna, Italy
Coordinating Investigator and address:	Marco Gambarotti MD Phone: 051-6366.652 Email: marco.gambarotti@ior.it
Methodology:	Experimental study with biological material (Single insitution case series review of clinical and histological data)
Type:	Academic
Founding:	None
Principal Investigator Signature	I confirm that I've read this protocol and I accept to run the study in compliance with what is stated in the protocol and with the ICh-GCP and all applicable law Marco Gambarotti MD Firma  _____

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BACKGROUND

One of the many diagnostic challenges in soft tissue pathology is the AFH – the Angiomatoid Fibrous Histiocytoma. It is a rare tumor of intermediate biological potential and uncertain differentiation accounting for approximately of 0,3% of all soft tissue tumors (Thway & Fisher, 2015). The AFH is a histiocytoid cell lesion with primary histological characteristics which include in the first place a lymphocytic cuff, a fibrous pseudocapsule and pseudoangiomatoid spaces with hemorrhage and hemosiderin deposition. Unusual but often present morphological features are also a sclerotic matrix, a myxoid stroma, the presence of osteoclasts and/or a reticulated pattern. Nuclear atypia, mitosis and eosinophils can also be found (Bohman, Goldblum, Rubin, Tanas, & Billings, 2014; Kao et al., 2014)

Its name still stems from when it was first described by Enzinger in 1979 as an 'Angiomatoid malignant fibrous histiocytoma' to acknowledge for its potential for metastasis and local recurrence and it was first classified as a malignant tumor although later on its proven actual relatively low risk for distant metastasis (<5%) was considered and the word 'malignant' was removed from its name, hence 'AFH' (Bohman et al., 2014; Thway & Fisher, 2015). Clinically, its true potential for local recurrence is being estimated to be as high as 15% (Thway & Fisher, 2015).

Although the tumor cells stain in a higher percentage for Desmin (50-92%), EMA (50-60%), CD68 (33-80%) and CD99 (up to a 100%), those markers are very little specific and molecular techniques such as FISH and PCR are recommended to make the diagnosis of an AFH with either one of the three translocations EWSR1-CREB1 (72%); EWSR1-ATF1 (21%) and FUS-ATF1 (7 %) being almost pathognomonic even though other tumors such as Clear cell sarcoma, Primary pulmonary myxoid sarcoma or the Hyalinizing clear cell carcinoma of the salivary gland harbor these mutations as well (Antonescu et al., 2007; Bohman et al., 2014; Chen et al., 2011; Kao et al., 2014; Rossi et al., 2007; Tanas et al., 2010; Thway & Fisher, 2015). There is a certain number of AFH which are negative for all rearrangement with FISH and it is being suggested that cryptic rearrangements of these genes undetectable by FISH or the existence of other translocations involving other genes account for these cases (Tanas et al., 2010; Thway & Fisher, 2015). Most likely FISH doesn't allow for detection of rearrangement due to the presence of submicroscopic insertions or because the genetic material simply is undetectable to the commercial break-apart probes (Thway & Fisher, 2015). RT-PCR is recommended to be performed in conjunction with FISH and even though FISH with break apart probes is faster and according to some more sensitive RT-PCR is far more specific as it also

identifies the fusion partner (Noujaim et al., 2017; Thway & Fisher, 2015). Useful negative immunohistochemical markers are Cytokeratins, S100, CD31 and Myogenin (Bohman et al., 2014).

AFH are most often found in the dermis or the subcutaneous tissue of the extremities of children and young adults. But it has a wide age range (infants – adults in the 9th decade) and a part from other somatic localizations such as trunk, head and neck non-somatic locations have also been reported such as lung, mediastinum, retroperitoneum, vulva, omentum, bone and ovary (Chen et al., 2011; Shi et al., 2015; Tataroglu, Culhaci, & Cecen, 2015; Thway & Fisher, 2015; Thway, Stefanaki, Papadakis, & Fisher, 2013).

Differential diagnosis for the AFH are tumors or lesions such as inflammatory myofibroblastic tumor, follicular dendritic cell sarcoma, poorly differentiated carcinoma, meningioma, lymph node metastasis, hematoma, granulomatous inflammation, myoepithelioma, schwannoma, primary pulmonary myxoid sarcomas, Ewing sarcoma, rhabdomyosarcoma, malignant rhabdoid tumor or aneurysmal fibrous histiocytoma (Chen et al., 2011; Kao et al., 2014; Shi et al., 2015; Thway & Fisher, 2015).

Recent case reports and studies of the metastatic potential of theses tumors have raised the question of and the necessity for a molecular or immunohistochemical marker or specific histological criteria that could correlate with the worse outcome for this particular group of patients. According to those studies metastasis was found in regional lymph nodes, bone and lung (Martinez, Moreno, Vinson, Dodd, & Brigman, 2016; Tay et al., 2016; Thway et al., 2013).

Also, in a recent study Cheah et al. reported on the results of ALK-expression of their series of eleven AFHs which showed that ALK expression in those tumors is common which had initially caused misdiagnosis as inflammatory myofibroblastic tumor which is one of the differential diagnostics of AFH (Cheah et al., 2018).

OBJECTIVE OF THE STUDY

Primary Objective

Primary objective is to present our series of AFH's with its clinical, histo-morphological and molecular characteristics of these tumors, in order to better understand their characteristics and to identify the best therapeutic approach.

Secondary Objective

Secondary objective is to reproduce the results of ALK expression from the study of Cheah et al. on our series of 9 AFH's cases.

STUDY DESIGN

This is single institution cases series review of histological and clinical data

POPULATION

Inclusion criteria

1) Male and female patients treated at Rizzoli Institute from 01 January 2006 to 31 December 2017
2) Diagnosis of Angiomatoid Fibrous Histiocytoma
3) Histological slides/formalin-fixed paraffin-embedded tissue tumor (FFPE) blocks from archive available to perform the histology analysis
4) Written informed consent prior to any study-specific analysis and/or data collection

Exclusion criteria

1) Patients with histological diagnosis different from Angiomatoid Fibrous Histiocytoma

MATERIAL AND METHODS

We will retrieve from the archives of the Rizzoli Institute all the cases with a histological diagnosis of Angiomatoid Fibrous Histiocytoma. By reviewing our database, we aspect to find approximately nine cases (actually all adults). We will review all the medical records, radiological imaging, and histological slides of all of these cases. In all cases with enough available material, immunohistochemistry will be performed on FFPE material. EWS-CREB1 and EWS-ATF1 mutation and ALK expression will be analyzed with RT-PCR and/or FISH analysis.

STATISTICS

To the case series will be applied a descriptive statistic.

ENROLLMENT PROCEDURE

Patients considered eligible will be included in the study, after providing a written informed consent.

Due to the incidence of mortality of the disease under investigation, it would be possible that some eligible subjects will be deceased**.**

DATA COLLECTION

Clinical data will be retrieved by patient charts.

A protocol-specific CRF reporting the results of the review will be provided.

A CRF is required and should be completed for each included subject.

ETHICS AND QUALITY ASSURANCE

The clinical trial protocol and its documents will be sent before initiating the study to the competent Authorities and Ethics Committees of each participating country for its approval.

The responsible investigator will ensure that this study is conducted in agreement with either the most updated Declaration of Helsinki and all the international and local laws that apply to clinical trials and to patient protection.

The protocol has been written, and the study will be conducted according to the principles of the ICH Harmonized Tripartite Guideline for Good Clinical Practice

(ref: http://www.emea.eu.int/pdfs/human/ich/013595en.pdf).

INFORMED CONSENT

All patients will be informed, by the investigator, of the aims of the study, the possible risks and benefits that will derive from the study participation.

The Investigator must clearly inform that the patient is free to refuse participation in the study and that can withdraw consent at any time and for any reason.

They will be informed as to the strict confidentiality of their patient data, but that their medical records may be reviewed for trial purposes by authorized individuals other than their treating physician.

The informed consent procedure must conform to the ICH guidelines on Good Clinical Practice. This implies that "the written informed consent form should be signed and personally dated by the patient or by the patient's legally acceptable representative".

The Investigator must also sign the Informed Consent form, and will keep the original at the site and a copy of the original must be handed to the patient.

The competent ethics committee for each Institution participating to the study must validate local informed consent documents before the study can be opened. It will be emphasized that the participation is voluntary and that the patient is allowed to refuse further participation in the study whenever he/she wants. This will not prejudice the patient's subsequent care.

Due to the high incidence of mortality of the disease under investigation, it would be possible that some potential eligible subjects will be deceased.

GENERAL PRINCIPLES FOR HUMAN BIOLOGICAL MATERIAL (HBM) COLLECTION

Human biological material (HBM) collection involves the collection and storage of biological material, residual biological material or derivatives in compliance with ethical and technical requirements.

Biological material (FFPE blocks of tumor sample) are already stored in the archive of the Istituto Ortopedico Rizzoli, Pathology Department.

The biological material will be used and stored according with the sample characteristic and applicable regulation.

- The Istituto Ortopedico Rizzoli will have a designated person responsible for collection and will act as a communication point
- The collected HBM should be documented, i.e. the amount remaining and its location. act as a communication point

CONFIDENTIALITY

In order to ensure confidentiality of clinical trial data as disposed the national and European applicable regulation, data will be only accessible for the trial Sponsor and its designees, for monitoring/auditing procedures, the Investigator and collaborators, the Ethics Committee of each corresponding site and the Health Authority.

Investigator and the Institution will allow access to data and source documentation for monitoring, auditing, Ethic Committee revision and inspections of Health Authority, but maintaining at all times subject personal data confidentiality as specified in the "Directive 95/46/EC of the European Parliament and of the Council of 24 October 1995".

The Investigator must guarantee that patient anonymity is kept at all times and their identity must be protected from unauthorized persons and institutions.

All patients included in the study will be identified with a numeric code, so that no identifiable personal data will be collected (pseudo anonymization)

The Investigator must have and conserve a patients' inclusion registry where it figures the personal data of the patient: name, surname, address and corresponding identification code into the study, this register will be kept on the Investigator File.

PUBBLICATION OF RESULTS

The results from this study will be published or shown at scientific conferences. The final publication of the study results will be written by the Principal Investigator.

SPONSOR ROLE AND RESPONSIBILITY

The sponsor is the sole owner of the data and is responsible of all the clinical trial activities from study design, development, data collection, management, analysis, interpretation of data, writing and the decision to submit the report for publication written by the Principal Investigator,

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