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**Guidelines and Recommendations**

# Procedures and operating instructions for diagnosis in vascular anomalies and pathology

L. Moneghini[1], M.C. Mihm[2], E. Fulcheri[3,4]

*[1] Unit of Pathology, Department of Health Sciences, San Paolo Hospital Medical School, University of Study Milan; [2] Harvard Medical School, Boston, MA, USA; [3] Fetal and Perinatal Pathology Unit, IRCCS Istituto Giannina Gaslini, Genoa; [4] Division of Anatomic Pathology, DISC, University of Genoa*

*Con l'approvazione di:*
*SISAV - Presidente Francesco Stillo*
*SIAPEC - Presidente Mauro Truini*

In the last 30 years a revolution has occurred in the diagnosis and management of vascular anomalies. The great changes began with Mulliken and Glowacki separation of hemangiomas and vascular anomalies. Their work has now morphed into the ISSVA classification. Subsequently the discovery of the significance of the presence of GLUT-1 in the diagnosis of the hemangiomas of infancy gave us a new marker in our quest for accurate classification. Now genetic breakthroughs have led us into a "Star Wars" like environment in the experimental laboratory. During all these events the critical role of the pathologist has become more evident. Understanding the histopathology of anomalies has greatly aided in our approach to therapies. Moreover, genetic findings do not have full significance without the morphologic framework

## Introduction

Histopathological examination of vascular anomalies and pathology represents a crucial moment in the diagnostic-therapeutic pathway in which the analysis of a sample becomes the confirmation or enrichment of information that the clinic and the instrumental examinations have already outlined and, at the same time, the starting point to trace further therapy.
It also allows to verify the adequacy and representativeness of the sample for the genetic examination, fundamental both for prognostic purposes and for the possible pharmacological treatment in order to guarantee the best quality of life for patients.
The recommendations for an adequate histo-pathological examination for vascular abnormalities also arise as a requirement to standardize the management and anatomo-pathological reporting for this type of pathology and to complete the guidelines for the diagnostic/therapeutic management of the same, already drafted by SISAV in the 2015 with a multidisciplinary approach, and in adherence to the ISSVA Classification for vascular abnormalities, which represents the current best international classification.

## Goals of correct histopathological examination by recommendations validated by a scientific society

I Define the exact nature of the lesion (diagnostic confirmations or ex novo diagnosis)
II Evaluate the exact tissue involved
III Verify the presence of proliferative foci in the lesion
IV Identify conditions of risk and complications
V Ensure the adequacy of the process (sampling,

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**Correspondence:** Laura Moneghini, Unit of Pathology, Department of Health Sciences, San Paolo Hospital Medical School, University of Study Milan, via Di Rudinì 8, 20142 Milan, Italy - E-mail: laura.moneghini@asst-santipaolocarlo.it

preparation and staining) for second opinion and biomolecular investigations

VI Ensure the preservation of material for any further investigations and case studies

## Operating instructions

1 What to send with samples:
- a histopathological examination request (minimum requirements);
- b preoperative iconographic documentation;
- c results of hematological analysis;
- d diagnostic imaging (CT, CT-angiography, MRI, Ultrasound, Doppler-ultrasound…);
- e report of previous treatments (embolisation, laser, pharmacological therapy, surgery…);
- f suitable material for further different investigations:
  - not fixed;
    - within one hour of sampling;
  - formalin fixed;
    - correlated by a sample in RNA later;
    - cryopreservation in biobanks;
- g blood sample in EDTA.

2 Fixation:
- a in buffered formalin;
- b minimum fixative volume 1:10;
- c cooled buffered formalin for large samples.

3 Sampling:
- a specimen orientation;
- b macroscopic photographic documentation;
- c possible “repere” in colored china;
- d serial sections, oriented and numbered progressively. Every sample smaller than 10 cm of major axis must be completely included.

4 Stainings:
- a haematoxylin/eosin;
- b histochemical staining:
  - i mandatory:
    - (a) Masson’s trichome;
    - (b) orcein;
  - ii not mandatory:
    1. Van Gieson;
    2. alcinan blu pH1;
    3. PTAH;
    4. Congo red;
    5. Azan-Mallory;
    6. staining for reticuline;
    7. Weigert elastic;
    8. alkaline phosphatase or Alizarin Red for calcium in cases suspect of calciphylaxis.
- c Immunocytochemical staining:
  - i mandatory:
    1. CD31;
    2. CD34;
    3. podoplanin;
    4. smooth muscle actin;
    5. WT-1;
    6. Ki 67;
  - ii not mandatory:
    1. Glut-1;
    2. Fli-1;
    3. VGFR;
    4. Lyve-1;
    5. PROX-1;
    6. S100 protein.

5 Molecular investigations:
Sec. ISSVA Classification 2014 (Rev. 2018).

6 Histopathological diagnosis must report:
- a vascular anomalies according to ISSVA Classification;
- b identification of the recognised vascular anomalies according to SISAV guidelines:
  - extension;
  - involvement of anatomical structures;
  - resection margins;
- c presence of additional components associated with vascular injury;
- d inflammatory state;
- e presence of proliferative, vascular and extravascular foci (i.e. “nidus”);
- f effects of the previously performed therapy (embolization, sclerotherapy, surgery…);
- g intercurrent diseases.

7 Biomolecular data:
- location of the mutation:
  - genetic;
  - somatic;
- type of mutation;
- percentage of mutated gene.

8 Preservation of the material:
despite having implemented the Guideline on “Traceability, collection, transport and storage of cells and tissues for diagnostic investigations”, published in May 2015 - SIAPEC, it is advisable to keep the residual material after sampling for at least 3 months after the scheduled date for disposal.

**Conflict of interest statement**

None declared.

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