Histopathological analysis of vascular malformations

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Short title: Histopathological analysis of vascular malformations

Abstract

Objective

To propose and develop a histopathological criteria to help diagnose vascular malformations

Methods

All patients who underwent surgical resection and had a confirmed histopathological diagnosis of vascular malformations from 01 March 2018 – 26 February 2020 were included. A criteria based on ten parameters was developed to help diagnose vascular malformations. Discrepancies between clinical and histopathological diagnosis were evaluated.

Results

A total of 18 cases were identified. There was a discrepancy between the clinical diagnosis and the initially reported histopathological diagnosis in 16 cases (88.9%). This was reduced to 7 (38.9%) and 6 cases (33.3%) with 1st and 2nd time revised histopathological analysis using proposed criteria.

Conclusions

The discrepancy between clinical and histopathological diagnoses of vascular malformations has highlighted the requirement of an agreed criteria for histopathologists to help formulate their diagnosis. The proposed criteria may be used as a guide in addressing this and guide treatment and improve clinical practice.

Keywords

Vascular malformations, Arteriovenous malformations, Vascular anomalies, Histology, Histopathology

Introduction

The classification of vascular anomalies has evolved over the past decade with many significant updates including their associated causative gene and histopathology. The International Society

of the Study of Vascular Anomalies (ISSVA) classification, which divides vascular anomalies into vascular tumors and vascular malformation, is widely used amongst clinicians and scientists but is primarily observational and clinical (1). Many pathologists do not routinely use existing classification systems such as the ISSVA (Figure 1) and therefore terminology will not be consistent with clinicians, resulting in discrepancy.

Traditionally, the role of histopathology for the management and/or diagnosis of vascular malformations has been very limited due to the lack of experience and interests from pathologists. Diagnosis is generally based on history and physical examination, along with radiological imaging. Therefore, routine biopsies are not performed, and typically histologic role has been confined to the differential diagnosis of malignant tumors. In addition, histopathologists is tasked to confirm or disagree with the clinical diagnosis and to classify malformations based on vessel type and presence or absence of histological evidence of arteriovenous shunting (2). Hence, there is a lack of basic histopathological guideline/criteria to assess and report the diagnosis of vascular malformations and pathologists typically use common histological features, as described above, along with clinical correlation, such as clinical history and physical examination, as a guidance. It is important to note that accurate histopathological diagnosis of various types of vascular malformations plays an important role in clinical management, for example among vasculsr malformations some respond well to sclerotherapy while others do not. However, it is essential that a meaningful interpretation of a specimen should involve review of both clinical information and imaging studies (3).

Recent advances in the understanding of association of vascular malformations with specific genetic mutations that may determine management plan are likely to increase the need of lesion

biopsy. This is likely to reenact the role of histological assessment complementing molecular analysis to improve the accuracy of diagnosis. Therefore, a standardized and objective histopathological criteria that complements ISSVA classification may help to improve the diagnosis accuracy. Therefore, the aim of the study was to propose and develop a histopathological criteria to help diagnose vascular malformations and retrospectively review cases of vascular malformations to determine accuracy of diagnosis and analysis of discrepancies, using the ISSVA classification as the gold standard for diagnosis of vascular anomalies.

Methods

Study design

We conducted a retrospective histopathological evaluation in a tertiary referral vascular anomaly center. This study was approved by the South-Central Berkshire research ethics committee (REC 19/SC/0090)

Patient selection

All patients who were clinically diagnosed as vascular malformations who underwent surgical resection and had a confirmed histological diagnosis as vascular malformations from 01 March 2018 – 26 February 2020 were included.

A total of eighteen cases were retrieved on hematoxylin and eosin (H&E) staining. The initial diagnosis of these cases are as described in Table 1.

Clinical diagnosis

Patients with suspected vascular malformation were assessed by a vascular anomalies multidisciplinary team consisting of two vascular surgeons and three interventional radiologists, and/or plastic surgeons. The clinical diagnosis of vascular malformation would be classified according to ISSVA classification based on an agreed consensus by the multidisciplinary team. The assessment was conducted by a standardized process including natural history, physical examination and medical imaging. Ultrasound duplex and/or magnetic resonance imaging was the preferred choice of imaging, used to assess the flow characteristics and characterize the lesion for treatment planning (4). Clinical diagnosis was obtained from medical notes after out-patient review. All patients had a single lesion with no genetic investigations undertaken for detection of any potential gene mutations.

Histopathologic diagnosis

The initial histopathologic diagnoses were reported by eight different consultant pathologists who did not use any criteria or classification such as the ISSVA.

As no histopathological criteria exists in diagnosing vascular malformations, a criteria, based on the current literature and local pathological specimens, was developed by a dermatopathologist (FD) with an interest in vascular anomalies, and a vascular biology clinician-scientist (CP). The histopathological criteria was developed with the aim to guide non-specialist pathologists while complementing ISSVA classification. This is important to ensure that such histopathological criteria would standardize and guide even non-specialized pathologists in assessment and reporting of the histopathological analysis of vascular malformations independently and accurately without deviating from ISSVA classification.

Based on this criteria, a revision of histopathological diagnoses was performed by one dermatopathologist (FD). This pathologist acted as the intra-observer and a standardized assessment process was undertaken in which the same parameters (as below) were evaluated for each histological specimen. In addition, the pathologist was blinded for both the clinical diagnosis and initial histopathologic diagnosis, and re-examined all tissue sections twice at five months apart. This was not the initial planned time frame but due to COVID-19 pandemic commitments, this had to be inevitably delayed. A second pathologist was initially recruited to perform the standardized assessment blindly and independently for the similar period to assess inter-observer variability although this was abandoned due to re-deployment of staff and personal reason during the COVID-19 pandemic.

The histopathological criteria was based on ten parameters:

1. Dermis/subcutis involvement
2. Lesion size
3. Presence of inflammation (0-3)
4. Presence of fibrosis (0-3)
5. Supporting structure
6. Vessel diameter
7. Wall thickness
8. Presence of thrombosis (0-3)
9. Major element of lesion (vein, capillary, lymphatic or artery)
10. Presence of pericytes (0-3)

In terms of parameters 3, 4, 8 and 10 of the criteria this was graded from a scale from 0 to 3, with 0 being no presence, 1 = mild, 2= moderate, 3 = severe.

These 10 parameters were chosen based on the current understanding of the pathogenesis of vascular malformations. For example, histopathological lesions of capillary malformations show enlarged venule-type channels with thickened walls. These channels extend into the deep dermis, subcutis and skeletal muscle (5). Venous malformations consist of an abnormal venous network of dilated vascular spaces that contain thin-walled channels with fibrosis, regions of thrombosis and phleboliths (6) (Figure 2). Meanwhile, AVMs typically showed arterioles, capillaries, and venules within a dense fibrous background combined with large arteries and thick-walled veins, and no evidence of thrombosis (7) (Figure 3 and 4). Inflammation has shown to play a major role in vascular dysmorphogenesis by weakening of vessel wall, leading to vascular instability and potential rupture (8,9). Multiple inflammatory and cytokine genotypes have been associated with clinical course demonstrating it as a key element in disease pathogenesis or progression (10). Localized intravascular coagulopathy has been associated with vascular malformations causing pain and thrombosis within a lesion (10,11). Pericytes function to stabilize the vessel and suppress endothelial cell proliferation, differentiation and migration and therefore the reduction in pericytes is thought to be associated in the pathology of vascular malformations (12,13). The dermis/subcutis involvement and lesion size is purely to appreciate the morphology of the lesion. Vascular malformations are extremely diverse and can range from small, superficial lesions to large, deeper lesions. The presence of inflammation, fibrosis and thrombosis can enhance our understanding of the pathogenesis of the lesions, however, this could be simply attributed to the trauma to the lesion and hence does not provide a true presentation of the biology of the vascular anomaly. Traditional histopathological interpretation of vascular malformations, in general, will

show an abnormal arrangement of blood vessels. However, depending on the type of malformation, specific features may be observed e.g. microcystic lymphatic malformations are characterized by dilated small vessels lined by a single layer of flattened endothelium, surrounded by rare pericytes and little or no smooth muscle (3,5,14). In comparison, these new 10 parameters of the proposed criteria is designed in a simplistic manner to supplement the existing terminology but most importantly enable institutions where local expertise is lacking to confidently approach the diagnosis of vascular malformation using this as a guidance. From the current understanding of the pathogenesis of vascular malformations and application of the proposed criteria, we can predict that histological assessment will often show limited pericytes, thrombosis and fibrosis. In addition, we expect vascular malformations to involve deeper into the skin layer i.e. the subcutis. However, inflammation may be more evident on histology. Furthermore, LFVM will often show a smaller vessel diameter and wall thickness in comparison to AVM. Nevertheless, these parameters will be dependent on the type of vascular malformation and the progress of the lesion.

Discrepancy

The study was conducted in the following order:

1. Cases identified (n=18) with histological diagnoses
2. New criteria developed
3. Histology reviewed and re-diagnoses with new criteria (1st time), noting any discrepancies
4. Histology reviewed again (five months apart) with new criteria (2nd time), noting any discrepancies

5. Clinical diagnoses reviewed noting any discrepancies
6. Intra-observer variability. This was assessed at different time points by the same observer to measure differences with the aim of evaluating the reliability and reproducibility of the new criteria.

In each case a discrepancy was defined as a difference in opinion between diagnosis whether this was a difference in terminology or a lack of vascular malformation subtype.

Staining

In cases where a diagnosis of an AVM was made, some tissue sections were stained for Elastin van Gieson (EVG) to confirm the initial presumption that the lesion demonstrated an arterial element (Figure 5).

Results

We identified a total of 18 cases in which a therapeutic surgical resection was performed. In all cases, tissue was sent to the pathology laboratory for histopathological examination. Characteristics of all cases are shown in Table 1.

Discrepancy between clinical and histopathological diagnosis

Before devising a histopathological criteria to help diagnose vascular malformations, clinical and initial histopathological diagnosis were compared for any discrepancy (Supplementary table 1). There was a discrepancy between the clinical diagnosis and the initially reported histopathological diagnosis in 16 cases (88.9%).

Discrepancy between clinical, initial and revised histopathological diagnosis

There was a discrepancy between initial, and 1st and 2nd time revised histopathological diagnosis in 16 cases (88.9%**)** and 11 cases (61.1%) respectively (Supplementary Table 1). The revised histopathological diagnosis is more specific in the subtypes of vascular malformations which are more closely in line with the ISSVA classification. In comparison, the initial histopathological diagnosis was largely reported as vascular malformations with subtype diagnosis lacking. In addition, many diagnoses were reported as AVM, which is presumed to be used as a broad term for vascular malformation and therefore inaccurate terminology, although often used by the clinician when populating the histology request form.

There was a discrepancy between clinical and 1st and 2nd time revised histopathological diagnosis in 7 cases (38.9%) and 6 cases (33.3%) respectively (Supplementary Table 1). However, it should be noted that the terminology used clinically is broader and typically classified as either low-flow or high-flow vascular malformations and therefore a subtype diagnosis is often lacking. Therefore, if a histopathological diagnosis of either venous, capillary, lymphatic or in combination was made but there was a clinical diagnosis of low-flow vascular malformation, this was not regarded as a discrepancy.

Figure 5 demonstrates the histological assessment of a late 50s male who presented with an expanding and painful lesion in the right upper eye lid causing drooping. Clinically this was diagnosed as a low-flow vascular malformation however, histologically this was diagnosed specifically as a combined low-flow vascular malformation. In figure 6, this is a late 60s female who presented with an expanding and painful lesion at the base of the right neck. MRI findings demonstrated a very large vascular malformation in the right side of the neck with evidence of

bony involvement, intracranial extension and large feeding and draining vessels. Clinically this was diagnosed as a low-flow vascular malformation but histologically this was diagnosed specifically as a venous malformation.

Intra-observer variability

There was a discrepancy in 9 cases (50%) between the intra-observer 1st and 2nd time (Supplementary Table 2 and Supplementary Table 3-4).

The histopathological criteria has shown that the presence of inflammation, fibrosis, thrombosis and pericytes may help in identifying vascular malformations. In 17 cases of vascular malformations (based on review diagnosis of intra-observer 2nd time) inflammation, fibrosis, thrombosis and pericytes was present in three (17.6%) cases. In addition, when comparing lesion size, vessel diameter and wall thickness, there was no correlation between in terms of lesion size between LFVM and AVM. However, 15 cases (93.8%) of LFVM lesions showed <10 mm and <0.5 mm for vessel diameter and wall thickness respectively. In comparison all AVM lesions showed ≥10 mm and ≥0.5 mm for vessel diameter and wall thickness respectively.

Discussion

In more than 80% of the cases in this study, there was a discrepancy between the clinical and initial histopathological diagnoses of vascular malformations. This may be partly due to the initial histopathological diagnoses were reported by non-specialized pathologists and there was no histopathological protocol or criteria in diagnosing vascular malformations hence, diagnosis was subjected to a wide spectrum of subjective interpretation. A reviewed diagnosis based on our proposed histopathological criteria significantly reduced the discrepancy between clinical and

histopathological diagnosis to less than 50%. This was partly due to our criteria helped in differentiating vessel types in vascular malformations (2, 4).

It should be noted that comparison between initial and revised histopathological diagnosis and clinical and revised histopathological diagnosis, the discrepancy was reduced further after second reading by the same observer. This was demonstrated in the results section where initial histopathological diagnosis reduced from 88.9% to 61.1% after second observation by intra-observer. This was also shown between clinical and histopathological diagnosis where discrepancy was reduced from 38.9% to 33.3% after second observation by intra-observer. This could be explained by the pathologist improved with experience. However, we appreciate that despite the improvement, there was still a discrepancy in nearly half the cases between clinical diagnosis and histopathological diagnosis despite a second read. This highlights the need for histopathological guidance/criteria to assist pathologists in diagnosis and as experience builds up, the discrepancy would be expected to reduce further. This study provided a preliminary criteria that has the potential to further improve the histopathological diagnosis and reduce its discrepancy with the clinical findings. Our future studies will focus on refining and improving the histopathological criteria further based on the ISSVA classifications.

A study by Mathes *et al* (15) showed that only 22% of the patients had a correct diagnosis before being referred to the vascular anomalies clinic and a study by Aronniemi *et al* (16) demonstrated that the histopathological diagnosis of 13 out of 15 patients did not correspond with the initial clinical diagnosis. These studies show that significant confusion still exists regarding the appropriate terminology and the need for correct diagnoses to identify subgroups which may

respond to specific therapy. Such discrepancy can also be explained by the differences that are encountered within a clinical and laboratory setting. The pathologists often observed minor histological capillary, lymphatic or arterial components within a lesion and hence concluding a diagnosis of combined low-flow vascular malformations. However, these minor elements are extremely difficult to identify on physical examination or imaging. Clinicians might diagnose vascular malformations as either low-flow or high-flow with limited subclassification of vessel type. This was also evident in a study by Al Adnani *et al* (17) which showed that 30% of lymphatic malformations and 100% of lymphovenous malformations were previously classified as vascular malformations. The authors also concluded that the ISSVA classification provided a useful framework for histopathologists to classify vascular anomalies but dependent on the adequacy of clinical information provided and the requirement of immunohistochemical staining. Interestingly, from the proposed histopathological criteria, the results demonstrated that the presence of inflammation, fibrosis, thrombosis or pericytes was limited in all types of vascular malformations. This could potentially be a helpful negative microscopic feature when distinguishing vascular malformations. In addition, vessel diameter and wall thickness correlated with the type of vascular malformation, whereby LFVM demonstrated a smaller vessel diameter and wall thickness in comparison to AVM. This suggests that it is worthwhile in measuring these parameters when analysing vascular malformations to distinguish the types. Pericytes are cells supporting endothelial cells that line the smallest diameter blood vessels and play a crucial role in maintaining and regulating endothelial cell structure. Aberrations in pericyte-endothelial cell interactions are associated with pathological conditions such as fibrosis and cancer. It is therefore not surprising that these are not seen as a microscopic feature in vascular malformations. We appreciate that some institutions may lack local expertise in vascular malformations. However,

the proposed histopathological criteria is designed with simple parameters that are easily followed by any trained pathologist. Nevertheless, a possible option to help in defining diagnosis is to provide standard histology slides, with annotations, of confirmed diagnosis as a reference point. Further enhancements in future proposed histopathological criteria that could be considered would include staining markers for cell types associated with vascular biology such as pericytes and endothelial cells. In addition, staining for common molecular markers such as PIK3CA, but also for markers associated with inflammation and angiogenesis. This would improve understanding of the pathophysiology of vascular malformations and lead to precision treatment for individual patients.

This study has several limitations. Firstly, the sample size of patients was small, and some might argue that this was a case series. In the authors' opinion, this study was more than a case series because it preliminarily evaluated a newly developed histopathological criteria against a retrospectively collected patient samples with the aim to improve the criteria further that would be later tested in a larger prospective trial. Secondly, since only patients who underwent surgical excision were included in the study, potential selection bias might have been introduced. It is likely that in future, there will be increase in biopsies of lesions being done for genetic analysis to guide targeted therapy for vascular malformations. Therefore, the importance and frequency of histological assessment of lesions suspected of vascular malformations are likely to increase with biopsies, and complement genetic analysis to improve management. Thirdly, there was a lack of immunohistochemical markers used which is essential in aiding diagnosis and particularly helpful in distinguishing different subtypes of vascular malformations. For example, D2-240 is a highly sensitive and specific marker for lymphatic endothelium (18,19) and therefore present in lymphatic malformations. In AVM, it can be difficult to distinguish between veins and arteries,

however, with the use of Verhoeff van-Gieson and orcein stains elastic tissue of arteries and arterioles can be demonstrated and therefore support the diagnosis of AVMs (20). Finally, the time period between 1st and 2nd reading by the intra-observer was five months apart and despite results showing a marginal improvement in reduced discrepancy, ideally the time frame should be shortened as the pathologist was 'deskilled' and potentially the learning benefit has somewhat diminished. On the contrary, it may suggest that the criteria was difficult to adhere or replicate and highlights the ambiguity of the criteria , in particular the major element of the lesion (vein, capillary, lymphatic artery). This was particularly difficult to interpret with the lack of immunohistochemical markers and also dependent on the quality of sectioning which should be included in future studies to refine the histopathological criteria. Since this study has shown the proposed histopathological criteria was able to differentiate vascular tumors and malformations, future work should focus on refining the assessment and reporting of both types of lesions separately in accordance to the ISSVA classification to reduce the complexity.

Reproducibility is important for the development of such histopathological criteria. The emphasis should be first to ensure the reproducibility by a single observer which was addressed in this study through intra-observer variability assessment. Unfortunately, we regretted that we were unable to complete our inter-observer assessment despite partially conducted the analysis owing to our second histopathologist who was available initially but left subsequently, partly due to COVID-19 pandemic re-deployment and personal reasons. We would however, aim to address this in future study on validating and refining the criteria with larger sample size. Nevertheless, within the criteria there was good reproducibility to parameters dermis/subcutis (88.9%), structure support (72.2%), inflammation (83.3%), thrombosis (72.2%) and pericytes (88.9%). Therefore, these particular parameters may be useful in aiding diagnosis.

Conclusion

Histopathological analysis of vascular malformations often lacks accuracy and consistency when compared to the clinical and radiological assessment of vascular malformations. This is evident from the discrepancy shown between clinical and histopathological diagnoses of vascular malformations in this study. This has highlighted the requirement of an agreed criteria for histopathologists including the non-specialists to help assess and report the diagnosis of vascular malformations accurately and in accordance to the ISSVA classification. With the use of the proposed histopathological criteria, the discrepancy was reduced from 89% to 33%. However, this may lead the way in introducing artificial intelligence (AI), as an emerging discipline, to increase both accuracy and high-quality care to patients by potentially combining morphology and radiological imaging data and incorporating this into AI. However, with AI assisted diagnosis in the near future with digital pathology an accurate criteria would be required, which this proposed criteria could feed into. However, further studies to improve and validate the proposed histopathological criteria with larger samples will be needed.

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Declaration of conflicting interests

The authors declare that there is no conflict of interest

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