ORIGINAL ARTICLE

Histopathological reporting of paediatric cutaneous vascular anomalies in relation to proposed multidisciplinary classification system

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Background: The terminology applied to vascular anomalies has been variable in previously published literature making interpretation suboptimal. The International Society for the Study of Vascular Anomalies (ISSVA) has proposed a revised classification based on clinical features and histopathological findings. This classification is increasingly being accepted as clinically useful and a platform for future studies.

Aims: To examine the extent to which the ISSVA classification can be practically applied to diagnostic histopathological specimens.

Methods: Cutaneous vascular lesions received in a single paediatric pathology unit during a 2-year period (2004–5) were reviewed, including glucose transporter protein 1 (GLUT1) immunostaining where required, and lesions were reclassified according to the ISSVA classification.

Results: 144 specimens were identified. Appropriate full clinical information was provided in only 17% of cases at submission. Infantile haemangiomas comprised 46% of cases, 18% of which were regressive type, initially inaccurately identified as vascular malformations before GLUT1 immunostaining. 30% of lymphatic malformations and all lymphovenous malformations were previously classified as vascular malformations, not otherwise specified.

Conclusions: The ISSVA classification of vascular anomalies provides a useful framework for histopathologists to classify vascular anomalies. However, meaningful and appropriate use of such a system is dependent on the adequacy of clinical information provided and routine use of immunohistochemical markers.

Vascular anomalies are disorders of aberrant vasculogenesis, angiogenesis or lymphangiogenesis. They are among the most common paediatric abnormalities, occurring in about 1% of children.¹ Despite their frequency, there remains confusion among both clinicians and pathologists regarding their terminology^{2–3}; one name may be applied to different entities and, conversely, the same lesion may be assigned to different categories by different health-care professionals. Such terminological confusion may have contributed to both suboptimal clinical management and research in this area.

Around 20 years ago a biological classification system for paediatric vascular anomalies based on physical findings, natural history and histopathological features was published.⁴ In this system, vascular anomalies were essentially divided into two broad categories of haemangiomas and vascular malformations (table 1). Haemangiomas represented tumours, characterised by rapid growth during infancy followed by gradual involution, their underlying pathophysiology being abnormal endothelial cell proliferation. By contrast, malformations represent lesions present at birth (although not necessarily clinically apparent at this time), which grow proportionally with the child and do not spontaneously regress, their pathophysiological basis being dysplastic vascular channel formation. Vascular malformations have subsequently been further subcategorised according to the predominant type of vascular channel (capillary, venous, arterial, lymphatic or a combination) and their haemodynamic features (low or high blood flow).^{4–6}

This simple classification represented a major advance in the understanding of vascular anomalies and provided a framework that would enable professionals who deal with these lesions to use a common terminology. In 1996, the

classification was adopted and expanded by the International Society for the Study of Vascular Anomalies (ISSVA; <http://www.ISSVA.org>).^{7–9} In this revised classification, vascular anomalies are similarly divided into tumours and malformations, thus reflecting the awareness of other vascular lesions that are also characterised histologically by endothelial cell proliferation, such as lobular capillary haemangiomas and tufted angiomas (table 2). However, the term “haemangioma of infancy” or “infantile haemangioma” has replaced the classic “haemangioma” described in the original classification and is now included in the group of tumours. Further, it has now also been reported that the erythrocyte glucose transporter protein 1 (GLUT1) is strongly and diffusely expressed in the lesional endothelial cells of infantile haemangiomas during all stages of their natural history¹⁰ but not in other vascular tumours or malformations and this finding has also been incorporated into the ISSVA classification.⁸

Accurate classification of vascular anomalies has major implications for management in terms of treatment and prognosis. Infantile haemangiomas are usually managed conservatively and occasionally treated with steroids, interferon α or vincristine. The treatment of vascular malformations ranges from laser treatment for capillary malformations to sclerotherapy and surgery for lymphatic and venous (slow blood flow) malformations and embolisation and surgery for arteriovenous (high blood flow) malformations.⁹

Despite the increasing general acceptance of the ISSVA classification, uncertainty in clinical use continues.^{3 11} For example, haemangioma continues to be used to generically

Abbreviations: GLUT1, glucose transporter protein 1; ISSVA, International Society for the Study of Vascular Anomalies

describe an array of vascular anomalies that in reality are malformations, and there are no publications based on histopathological studies assessing the value of the proposed ISSVA classification for use by diagnostic pathologists. In this study, we applied the ISSVA classification to all cutaneous vascular anomaly specimens received during a 2-year period to assess whether it can be reasonably applied to routine diagnostic histopathology specimens.

MATERIALS AND METHODS

All cutaneous vascular anomaly specimens received during 2004–5 were reviewed and reclassified according to the ISSVA classification. In addition, the adequacy of clinical information provided by the submitting clinical team was assessed (particularly regarding presence or absence of the lesion at birth, location, size, appearance, progression and results of imaging studies). The original sections stained with haematoxylin and eosin and the immunostained sections were reviewed and, if not already performed, sections from all cases in which any small thin-walled vascular channels were apparent raising the possibility of infantile haemangioma, were immunostained for GLUT1 using a standard immunohistochemistry protocol. In brief, sections were dewaxed and rehydrated. Antigen retrieval was performed by pressure cooking. The GLUT1 antibody used was rabbit antihuman GLUT1 (Dako, Ely, Cambridgeshire, UK) at a 1:500 dilution, and bound antibody was detected using a streptavidin–biotin complex technique (ChemMate EnVision Detection Kit, Dako). Sections from placental tissue were used as a positive control and negative controls consisted of tissue sections from each specimen processed in parallel with omission of the primary antibody. In addition, immunostaining for lymphatic channels using D2-40 antibody (Dako) at a 1:100 dilution was carried out in morphologically selected cases using the same technique as GLUT1 staining. Sections from a clinically and radiologically confirmed neck lymphangioma (cystic hygroma) were used as positive controls. The final review ISSVA diagnosis was compared with the original reporting diagnosis, which was based on modifications of the original, simplified classification (table 1), as was the departmental protocol during this period. The study was approved by the local research ethics committee.

RESULTS

In all, 144 cases of cutaneous vascular anomalies were identified during the study period, representing approximately 2% of departmental surgical specimens. Adequate clinical information to allow incorporation of all relevant clinical and radiological data was provided on the histopathology request form in only 17% (25/144) of cases. All cases could be routinely subclassified and table 3 shows the ISSVA classification of all cutaneous vascular anomalies reported during the study period.

Table 1 Original classification of vascular anomalies, based on Mulliken and Glowaki's⁴ classification

Haemangioma	Vascular malformation
Proliferative phase	Simple
Involutive phase	Capillary
	Venous
	Arterial
	Lymphatic
	Combined
	Capillary venous
	Arteriovenous

Infantile haemangiomas represented 46% of cases (fig 1). Most of these were easily apparent on initial histopathological assessment and were confirmed by positive GLUT1 immunostaining. However, on haematoxylin and eosin staining, 18% (12/66) of these cases initially seemed to represent vascular malformations, with large dermal vessels, but represented involuted or late-stage infantile haemangiomas, with scanty residual GLUT1-positive lesional capillaries highlighted after immunostaining (fig 1).

Thirty per cent of lymphatic malformations (fig 2) and none of the lymphovenous malformations had been previously fully subclassified, these cases were initially reported as vascular malformation, not otherwise specified; and in most of these cases, no adequate clinical history was provided.

DISCUSSION

Our findings have shown that the proposed ISSVA classification of cutaneous vascular lesions can be simply and routinely applied to diagnostic histopathology specimens. However, routine immunohistochemical staining is required in a large proportion of cases for their correct subclassification, and adequate clinical information should also be provided. The management of vascular anomalies is a rapidly developing discipline that requires a multidisciplinary approach from doctors, surgeons, radiologists and histopathologists. No single specialist has sufficient expertise to diagnose and treat vascular anomalies in all the organ systems,¹² and accurate subclassification of the lesions is also important for the correct interpretation of future studies regarding natural history and management. In a study of referrals to a vascular anomalies multidisciplinary clinic, only 22% of the patients had been assigned a correct specific diagnosis before being referred.¹³

An issue encountered in reporting vascular anomalies in general, and applying the ISSVA classification in particular, was the adequacy of clinical information provided on the pathology request forms (only 17% of cases in our series provided full information). Nakhleh *et al*¹⁴ reported that <5% of cases submitted to a group of surgical pathology departments had inadequate clinical information; however, the definition of “adequate” in this study was simply any provision of a suggested clinical diagnosis or differential diagnosis. Similarly, Burton and Stephenson,¹⁵ who also defined adequate clinical information as any history or differential diagnosis, reported an inadequacy rate of <10%. However, for the purposes of the ISSVA classification, a fully adequate clinical history with respect to vascular lesions should include presence or absence of the lesions at birth, location, size, appearance and progression of the lesion, as well as results of imaging and Doppler ultrasound, as all these features, in addition to the pathological findings, should ideally be considered by the pathologist before offering a specific diagnosis on a submitted specimen. In some cases, morphological histopathological appearances alone may be misleading.¹⁶ For example, infantile haemangiomas, lobular capillary haemangiomas, congenital haemangiomas and tufted angiomas may appear morphologically similar, especially on small biopsy specimens, and rendering a correct diagnosis without adequate clinical information can be difficult, even when immunostaining is performed. A possible solution would be that, in the absence of appropriate clinical history, the pathologist issues an interim report until the cases are discussed at a multidisciplinary team meeting. An alternative would be to use a vascular anomalies “proforma” containing fields for all the required clinical information before submission, which can also be filed in the medical record so that if another

Figure 1 Infantile haemangioma at different stages of its natural history. (A,B) Proliferative phase infantile haemangioma consisting of lobules of densely packed capillaries lined by plump endothelial cells. Glucose transporter protein 1 (GLUT1) immunostaining is strongly and diffusely positive in the lesional endothelial cells. (C,D) Involved or regressed infantile haemangioma with scattered large, thick-walled blood vessels and residual scanty GLUT1-positive lesional capillaries.

specialist sees the patient, it can be easily reviewed and updated.

Infantile haemangiomas are the most common vascular tumours in infancy, affecting 4–10% of children.^{10 12 17} They have a distinct clinical behaviour, histological appearance and immunohistochemical profile (GLUT1 immunopositivity).^{2 4 18} When the ISSVA classification was used, they formed 46% of our cases. Most were correctly diagnosed, except for a minority of cases that represented late-stage or involuted haemangiomas. These can be extremely difficult to differentiate from venous or arteriovenous malformations on the basis of haematoxylin and eosin histopathological staining alone.^{18 19} A suggestive clinical history, if available, would be useful, and the availability of GLUT1 immunostaining has now facilitated an accurate diagnosis of these lesions. A total of 91 cases had either a clinical suspicion or histopathological features raising the possibility of an infantile haemangioma. We propose that in the absence of adequate clinical history, histopathologists should perform GLUT1 immunostaining on all vascular anomalies that contain any possible capillary component, as the diagnosis of infantile haemangioma in equivocal regressing cases may

affect clinical management and provide reassurance for the parents.

The next most frequent category in this series was lymphatic and lymphovenous malformations. Clinically, these are the most difficult lesions to classify because of the difficulty in distinguishing them from haemangiomas confined to the subcutaneous tissue.²⁰ Imaging methods, if available, provide useful additional information to distinguish between haemangiomas and malformations in most instances.⁵ In the absence of adequate clinical history, it may also be difficult to distinguish lymphatic malformations and lymphovenous malformations, as there may be intralesional haemorrhage and the presence of an apparent smooth-muscle coat around larger vascular channels. Furthermore, the identification of a large proportion of lesional lymphatic channels requires specific searching for their presence. However, D2-40 is a recently available monoclonal antibody that is reported to be a highly sensitive and specific marker of lymphatic endothelium,^{21 22} which, if confirmed and introduced into routine practice in such cases, will be useful in the further classification of these vascular lesions.²³ As a result of our findings, D2-40 has now been added to the

Table 2 The current classification set by the International Society for the Study of Vascular Anomalies ^{7–9}				
Tumours	Malformations Simple		Combined	
Infantile haemangiomas (GLUT1 positive)	Slow flow	High flow	Slow flow LVM	High flow AVM
Congenital haemangiomas (RICH and NICH)				
Pyogenic granuloma (lobular capillary haemangioma)	Capillary Venous Lymphatic	Arterial	CLVM	CAVM
Tufted angioma				
Kaposiform haemangioendothelioma				
Haemangiopericytoma				
AVM, arteriovenous malformation; CAVM, capillary arteriovenous malformation; CLVM, capillary lymphovenous malformation; LVM, lymphovenous malformation; NICH, non-involuting congenital haemangioma; RICH, rapidly involuting congenital haemangioma.

Figure 2 Histological features of cutaneous vascular malformations. (A,B) Lymphatic malformation composed of irregular thin-walled vascular channels with occasional lymphoid aggregates in the intervening stroma. D2-40 immunostaining is positive in the lymphatic channels. (C,D) Venous malformation with irregular vascular channels containing smooth muscle in their walls. Immunostaining for D2-40 is negative.

immunohistochemical panel to be routinely performed in all such cases in our laboratory (fig 2).

The ISSVA classification is increasingly being accepted as clinically useful and as a framework for understanding the pathogenesis of vascular anomalies,^{3 8 11 20} but its application remains largely absent from the histopathology literature. In this study, we found the separation of vascular anomalies into tumours and malformations to be a useful step towards moving beyond the sometimes confusing terminology that is used in the existing pathology-based literature on this topic, and accurate subclassification is increasingly possible with the use of specific immunohistochemical markers. From a practical perspective, histopathologists can therefore influence clinical management, even in the absence of appropriate clinical information, by attempting to classify vascular anomalies into the categories proposed by the ISSVA, with further discussion at multidisciplinary meetings.

Finally, although the ISSVA classification seems to be useful, it also has limitations, particularly as associations

between vascular tumours and malformations have been reported in some patients.¹¹ In addition, vascular anomalies acquired after infancy in disorders such as Von Hippel Lindau syndrome cannot always be neatly placed into the existing ISSVA categories.³ Further, the pathological aspects of more recently described lesions such as rapidly involuting congenital haemangioma and non-involuting congenital haemangioma may be difficult to determine on the basis of limited biopsy material, although again in this setting, immunohistochemical staining is aiding this process, as both rapidly involuting congenital haemangioma and non-involuting congenital haemangioma are GLUT1 negative.^{18 19} However, from a diagnostic histopathology perspective, our study shows that the classification seems to be the best currently available, is applicable to routinely submitted pathology specimens and should be used by both clinicians and histopathologists whenever possible, recognising the limitations of dependency on appropriate clinical information.

Table 3 Summary of findings from 144 cases of vascular anomaly classified according to the International Society for the Study of Vascular Anomalies classification			
	ISSVA classification, n (%)	Previous histological classification using old system	Agreement between two classifications (%)
Infantile haemangioma	66 (46)	54	82
Pyogenic granuloma (lobular capillary haemangioma)	15 (10)	15	100
Tufted angioma	2 (1)	2	100
Venous malformation	6 (4)	4	67
Lymphatic malformation	37 (26)	26	70
Lymphovenous malformation	13 (9)	0	0
Arteriovenous malformation	4 (3)	0	0
Capillary lymphatic venous malformation	1 (1)	0	0

ISSVA, International Society for the Study of Vascular Anomalies.
The degree of agreement between the original classification and the ISSVA classification is also shown.

Take-home messages

- Vascular anomalies may be usefully classified according to the Issa schema.
- Routine classification of some paediatric lesions requires use of immunostains such as GLUT-1 and D2-40.
- Regressive infantile haematomas may be initially misclassified as vascular malformations if immunostaining is not carried out.

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ECHO

Sampling sites for HPV detection

Please visit the *Journal of Clinical Pathology* website [www.jclinpath.com] for a link to the full text of this article.

Human papillomavirus (HPV) is more readily detected in male external genitalia than in the urethra or meatus. A study to find which anatomical sites needed to be sampled to detect HPV in the lower male genital tract surveyed 820 Mexican soldiers, with a median age of 24 years (range 16-50 years).

All men gave samples collected using a cytobrush moistened in phosphate buffered saline to brush the penis from bottom to top, starting at the middle third of the scrotum. After retraction of the prepuce (for uncircumcised men), the coronal sulcus, the glans, and the tip of the penis were also brushed.

A second cell sample was collected from two groups within the study. In group 1, the sample was taken from the distal urethra by introducing a swab 2 cm deep. In group 2, the sample was obtained from the meatus urethralis by opening the tip of the penis and introducing a cotton swab 0.5 cm deep. The different samples were tested for 27 HPV types.

HPV DNA was detected more frequently in samples from the external genitalia (46.4%) than from the urethra (20.8%) or the meatus (12.1%). Lack of samples from the urethra or meatus would have led to 5.1% and 1.5% false negative results, respectively.

The most frequently detected high risk HPV types (HPV 59, 52, 51, and 16) were found in different sites whereas low risk types were rarely found in urethra samples. The additional cell sample from the meatus made a negligible contribution to HPV detection. The detection was improved by the addition of the urethra samples but the discomfort of the procedure may not justify the gain in large epidemiological studies.

▲ Aguilar LV, et al. *Sexually Transmitted Infections* 2006;**82**:31-33