Nusantara Medical Science Journal
Volume 10 Issue 1, January – June 2025
P-ISSN: 2460-9757, E-ISSN: 2597-7288
Nationally Accredited Journal, Decree No. 36/E/KPT/2019.

Original Article

Non-involuting congenital hemangioma have angioproliferative features of both congenital vascular malformations and of vascular tumors-insights from apoptosis, autophagy and senescence

Amalia M. Utami [1,2], Gonca Cinkara [1], Kartika Ratna Pertiwi [3], Max M. Lokhorst [4], Onno J. de Boer [1], Chantal MAM van der Horst [4], Lorine B. Meijer-Jorna [5], Allard C. van der Wal [1]

[1] Department of Pathology, Amsterdam University Medical Center-location AMC, University of Amsterdam, Amsterdam, The Netherlands
[2] Department of Pathology Anatomy, Faculty of Medicine, Hasanuddin University, Makassar, Indonesia
[3] Faculty of Medicine, Universitas Negeri Yogyakarta, Yogyakarta, Indonesia
[4] Department of Plastic Surgery, Amsterdam University Medical Center-location AMC, University of Amsterdam, Amsterdam, The Netherlands
[5] Symbiant Pathology, Noordwest Ziekenhuisgroep, Alkmaar, The Netherlands

Corresponding Author:
Name: Amalia M. Utami
Email: amaliautami@med.unhas.ac.id

ARTICLE INFO

Keywords:
Hemangioma; Vascular Malformations; Arteriovenous Malformations.

DOI:

ABSTRACT

Background: Non-Involuting Congenital Hemangiomas (NICH) are benign vascular lesions characterized by microvessel proliferation, yet their pathophysiology and relation to congenital vascular malformations remain unclear. This study aimed to explore age-related NICH discrepancies and compare them with vascular malformations by examining histological features and markers of cell death, maturation, and proliferation, to clarify their classification as vascular tumors or malformations.
Methods: We performed immunohistochemistry on 36 paraffin-embedded samples (12 NICH, 24 congenital vascular malformations). Samples were stained for C-Caspase-3, Ki67, p62, LC3B, p21, and p27, and double-

stained with smooth muscle actin. Three independent observers semi-quantitatively scored samples. Statistical analysis compared NICH in two age groups (younger vs. older) with congenital vascular malformations.
Results: Lesions were categorized into younger NICH (n=7), older NICH (n=5), non-proliferative (n=12), and proliferative (n=12) vascular malformations based on clinical history, histology, and Ki67. C-Caspase-3 and LC3B expression was significantly higher in younger NICH ($p<0.05$) and proliferative AVM ($p<0.05$). The senescence marker p21 was elevated only in proliferative AVM, while p27 showed higher expression in both NICH and proliferative AVM versus non-proliferative vascular malformations.
Conclusion: This study revealed distinct cell cycle-related protein expression patterns across benign vascular anomalies and within the same anomaly across age groups. Despite shared clinical traits with congenital vascular malformations, NICH exhibited persistent vasoproliferative behavior and unique apoptosis, autophagy, and senescence patterns. These findings suggest NICH should not be classified as congenital vascular malformations, though similarities with proliferative AVM warrant further investigation into the nature of proliferative AVM components.

1. INTRODUCTION

Non-Involuting Congenital Hemangiomas (NICH) are distinctive benign vascular lesions, fully formed at birth, that, unlike infantile hemangiomas, classicly fail to regress postnatally, instead growing proportionally with the patient (1). Histologically, NICH are characterized by large lobular collections of capillaries lined by plump endothelial cells, often accompanied by prominent interlobular fibrous tissue containing dilated or malformed arteries and veins (2). This peculiar vascular architecture, including the presence of abnormal shunting into extralobular veins, bears histological resemblance to arteriovenous malformations (AVMs) (3).

Clinically, NICH, along with Rapidly-Involuting Congenital Hemangiomas (RICH) and Partially-Involuting Congenital Hemangiomas (PICH), are categorized under the umbrella of Congenital Hemangiomas (CH) by the International Society for the Study of Vascular Anomalies (ISSVA) (4,5). Despite this overarching classification, the functional distinction among these subtypes is profound; RICH are known to undergo spontaneous and complete regression, whereas NICH definitively do not involute (6) (7). This stark difference in clinical behavior raises fundamental questions regarding the underlying pathophysiology of each entity.

Despite the established clinical classification of NICH, the molecular pathophysiology underlying their persistent growth and failure of postnatal regression remains critically understudied. A classificatory ambiguity persists for NICH; they exhibit vasoproliferative features akin to vascular tumors, such as infantile

hemangiomas (IH), yet simultaneously display similarities with congenital vascular malformations through their lifelong lack of regression and the presence of malformed vascular elements. The absence of glucose transporter-1 (GLUT-1) expression in NICH, a unique diagnostic marker for IH, further complicates classification efforts and underscores the need for deeper mechanistic understanding (8). The ongoing debate over whether NICH should be classified as true hemangiomas or as congenital vascular malformations highlights the crucial need to identify specific biological markers and cellular pathways that can definitively distinguish these entities and guide management strategies.

While fetal proliferation in NICH is acknowledged (9), the postnatal mechanisms governing their sustained growth and failure to regress, particularly those involving cell cycle-dependent proteins, apoptosis, autophagy, and senescence, have not been comprehensively characterized. The dynamic balance between cell proliferation and programmed cell death (apoptosis) is fundamental for normal tissue development and homeostasis. Prior studies have indeed investigated apoptotic activity in other vascular anomalies, even demonstrating increased apoptosis during the involution of certain vascular tumors (10–12). However, these investigations typically have not specifically focused on NICH or fully elaborated on the multifaceted roles of other cellular processes like autophagy and senescence within the context of NICH's non-involuting pathophysiology.

To address this critical knowledge gap, this study aims to comparatively investigate the expression of proteins involved in cell proliferation (Ki67), apoptosis (Cleaved Caspase-3), autophagy (p62 and LC3B), and senescence (p21 and p27). By analyzing these markers in NICH specimens from different age groups and comparing them with congenital vascular malformations, especially those exhibiting proliferative components, this study seeks to uncover fundamental differences in cellular behavior. The findings from this research are expected to provide robust molecular evidence to clarify the classification of NICH as either a persistent vascular tumor entity or a vascular malformation with distinct proliferative features, thereby potentially guiding the development of more precise diagnostic and therapeutic approaches in the future.

2. METHODS

Selection of study materials

Paraffin blocks of patients who were surgically treated for distinct types of vascular anomalies were selected from the archives of the Department of Pathology at Amsterdam University Medical Center (Amsterdam UMC), location AMC. Clinical diagnosis and corresponding histopathology of 36 patients resulted in a selection of cases who were diagnosed as 12 cases of NICH, 17 cases AVM, and 7 cases venous malformations (VM). In the NICH group, individuals younger than 7 years were classified as young ages, whereas those aged 7 years or old were categorized as old ages. Of the 12 NICH cases, seven were defined as young and 5 as old ages. Nine of them were males and 3 females. The vascular malformations group (AVM and VM) consisted of 12 males and 12 females

Ethical Clearance

Informed consent forms were sent by mail to patients or their families. Review Board of the Amsterdam UMC granted a waiver for the use of leftover materials after

surgery (reference number: W19_224#19.269). Project was further approved by the Pathology Biobank of AUMC.

Histology

Serially cut sections (4µm) were stained with Hematoxylin & Eosin (H&E) and Elastic van Gieson (EvG) respectively to confirm the diagnosis of NICH, AVM, or VM. Adjacent sections were mounted for IHC staining. Histology of NICH demonstrates lobules of proliferating capillary vessels. Endothelium often showed hobnail appearance. These lobules were surrounded by fibrous stroma, containing larger dilated muscular vessels. (1). Histology of AVM shows conglomerates of large, tortuous, arteries and thick-walled veins, surrounded by fibrous or fibromyxomatous stroma, containing arterioles, capillaries, and venules(13). AVM with a proliferative component show additional solid areas of closely packed capillary microvessels with plump endothelium. Histology of VM demonstrates convolutes of tortuous, often malformed dilated veins, lined by flat endothelium (14).

Immunohistochemical single staining

The following primary antibodies were applied: a rabbit monoclonal anti-GLUT-1 (ThermoFisher Scientific, Waltham, USA), a rabbit monoclonal anti-Ki67 (proliferating cells, ThermoFisher Scientific), a rabbit monoclonal anti-cleaved Caspase-3 (C-Caspase-3) (apoptosis, Cell Signalling Technology, Danvers, USA), a mouse monoclonal anti-p62 (autophagy, BD Bioscience, New Jersey, USA), a mouse monoclonal anti-LC3B (autophagy, Enzo Life Sciences, Brussels, Belgium), a mouse monoclonal anti-p21 (senescence, DAKO, Glostrup, Denmark), a mouse monoclonal anti-p27 (DAKO).

Single IHC staining for GLUT-1, Ki67, C-Caspase-3, p62, LC3B, p21, and p27 was applied on all cases. First step, sections were dewaxed in xylene and rehydrated in graded-alcohols prior to antigen retrieval with heat-induced epitope retrieval (HIER) in a PT Module (ThermoFisher Scientific, Waltham, MA, USA) using Tris-EDTA buffer (ThermoFisher Scientific). Then, endogenous horseradish peroxidase (HRP) was blocked in methanol + H2O2 (30% diluted in methanol into 0.3% solution). Incubation with primary antibody for GLUT-1, Ki67, Caspase-3, p62, LC3B, p21, and p27 for 60 minutes at room temperature, followed by HRP anti-mouse polymer secondary antibody (Brightvision, Immunologic, Duiven, the Netherlands) for 30 minutes. Vector Novared (Vector Laboratories, Burlingame,CA, USA) was used as a chromogen. Sections were counterstained with Hematoxylin, dried on a hotplate and coverslip sealed with Vectamount (Vector Laboratories). Next, sections were digitized using Philips IntelliSite UFS (Philips Digital Pathology Solutions, Best, the Netherlands) for further semi-quantitative analysis(15). Positive control tissues for IHC consisted of a paraffin block containing normal tonsil tissue.

Semi-quantitative analysis

To evaluate the expression of the performed immunostainings, a semi-quantitative scoring was developed, and performed by three observers independently (AMU, GC, and KRP). GLUT-1 expression of endothelial cells was scored as either positive or negative. Ki67, C-Caspase-3, p62, LC3B, p21, and p27 antibody reactivity was evaluated based on the percentage of vessels containing immunopositive cells. The scoring system was as follows: 0: negative, 1: 1 focus (1 or more positive cells in 1

cluster) (<25%), 2: from more than 2 positive cells in 1 cluster and 2 positive clusters (multifocal 25-50%), and 3: diffuse positive (>50%)(10).

Comparison of semiquantitative scores was analyzed between groups: NICH young versus NICH old (>7 years old), NICH versus congenital vascular malformations without proliferation (AVM and VM) and NICH versus vascular malformations with microvascular proliferation. In addition, we also compared vascular malformations with and without a proliferative component.

Statistical analysis

The results of IHC were counted as semi-quantitative scoring. Initially, a normality test was performed to identify the distribution of the data, and the results showed that the data were not normally distributed. Therefore, to compare immunopositivity expression of all antibodies between four groups, we used a non-parametric Mann-Whitney U test. P-values < 0.05 were considered as statistically significant. Statistical analysis was performed with SPSS 28.00 (IBM Corporation, Armonk, NY, USA).

Double Immunohistochemistry

Double IHC was performed to assess the cell-specific immunolocalization of p62, C-Caspase-3, and Ki67 antibodies within the margins of vascular walls by applying additional staining of the same sections with anti-smooth muscle actin-1 (SMA-1) antibody, reactive with vascular smooth muscle cells. We applied the sequential alkaline phosphatase (AP) double staining method with Vector Blue (Vector Laboratories) and Vector Red (Vector Laboratories) as chromogens. After tissue pre-treatment as described above, the first primary antibodies (P62, C-Caspase-3, or Ki67) were applied, followed by anti-mouse AP-conjugated polymers (Immunologic). The immunoreactivity was visualized in red using Vector Red. Then, the antibodies from the first staining were removed by a second HIER step using for 10 minutes, which left the Vector-red reaction product intact. After this treatment, we applied the second antibody (SMA-1), followed by anti-mouse AP-conjugated and visualized in blue using VectorBlue (Vector Laboratories). The double stained sections were mounted with Vectamount (Vector Laboratories).

3. RESULTS

Histology

Table 1. Characteristic of all 36 samples of NICH, AVM, and VM in this study.

Variable	n	%
Age (years) (mean ± SD)		**22.8 ± 21.7**
Diagnosis		
NICH young age	7	19.4
NICH old age	5	13.9
AVM proliferative	12	33.4
AVM non-proliferative	5	13.9
VM	7	19.4
Sex		
Female	15	41.7

Male	21	58.3
Location		
Neck	2	5.5
Forehead	2	5.5
Ear	2	5.5
Cheek	1	2.7
Back	1	2.7
Shoulder	1	2.7
Upper Extremities	2	5.5
Lower Extremities	4	11.1
Upperlip	1	2.7
Orbita	5	13.8
Eyebrow	1	2.7
Underlip	2	5.5
Gluteus	1	2.7
Skin	9	25
Subcutis	1	2.7
Bone	1	2.7

The diagnosis of the 12 selected cases of NICH, 17 cases of AVM, and 7 cases of VM was confirmed using H&E and EvG stainings (Table 1). IHC analysis of GLUT-1 in all samples yielded negative results, ruling out the diagnosis of IH. With the use of the proliferation marker Ki67 (16) on the vessels, the lesions were categorized into proliferative and non-proliferative vascular malformations. The non-proliferative vascular malformations group included 5 cases of AVM and 7 cases of VM, equally distributed over males and females. The proliferative vascular malformations group consisted of 12 cases of AVM, also equally distributed over males and females (Table 1).

Semi-quantitative analysis from single immunohistochemistry

A comparison of semi-quantitative scoring of immunostaining was performed among four groups: NICH young ages, NICH old ages, non-proliferative vascular malformations, and proliferative vascular malformations. The quantification results for the expression of Ki67, C-Caspase-3, p62, LC3B, p21, and p27 in these four groups are presented in Figure 1. Additionally, the IHC staining pattern is depicted in Figure 2.

Figure 1a compares the expression patterns in the young and old NICH age groups, revealing high proliferative activity of cells in capillary vessels in both cases. Cleaved Caspase3 expression was significantly reduced in the old age NICH samples. Additionally, the autophagy markers p62 and LC3B showed decreased levels in the old age NICH samples, with p62 being entirely absent in all old age NICH samples. The protein p21 exhibited low expression in both young and old NICH samples, whereas p27 was expressed at similarly high levels in both groups.

In Figure 2b, we compared the expression patterns between NICH (all ages) and vascular malformations without microvascular proliferations. Proliferation, as detected with Ki67, was significantly higher in the NICH cases. There were no differences in the apoptosis rate (cleaved Caspase-3) between the two groups. Among the autophagy markers, only LC3B showed high expression in NICH, and it was

significantly higher compared to non-proliferative vascular malformations. The expression of p21 was low, while p27 was high in both non-proliferative vascular malformations and NICH.

Figure 1c compares NICH and AVM samples with a proliferative component. Both show a similar high expression of Ki67 (proliferation), but cleaved Caspase3 is significantly increased in proliferative AVM. p62 was high in proliferative AVM and significantly increased when compared to NICH. There were no significant differences in LC3B expression. p21 and p27 were low and highly expressed, respectively, in both groups, without significant differences.

In addition, we also compared the expression of the cell activation markers between the two groups of vascular malformations (see fig 1d). Interestingly, expression of all investigated markers were significantly higher in the AVM with a proliferative component.

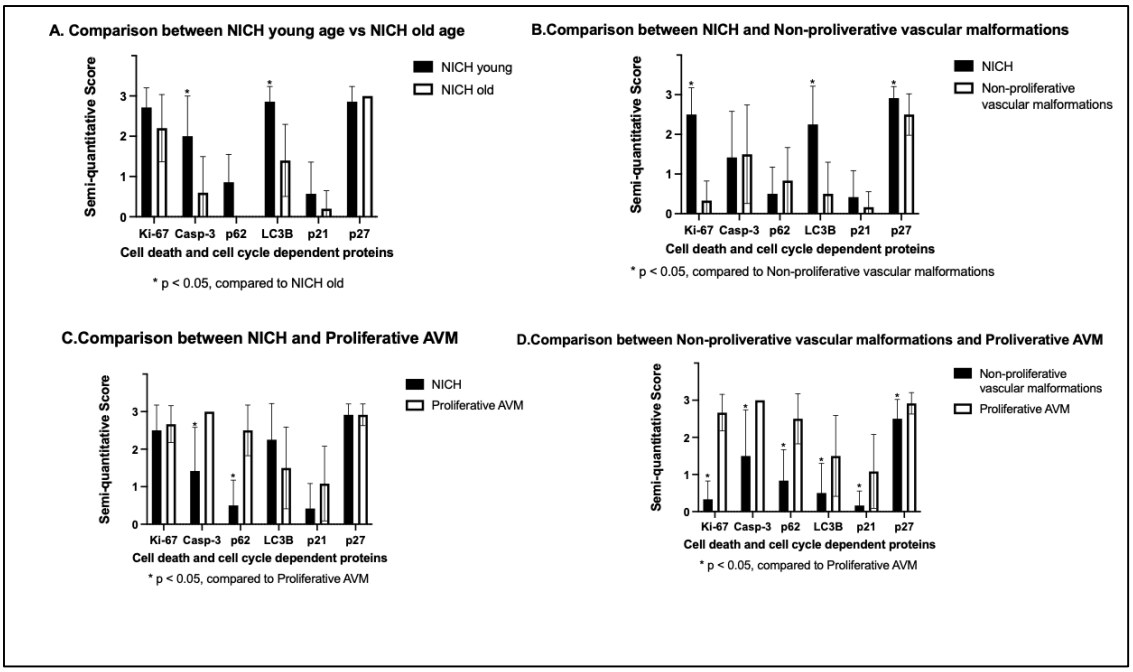

Figure 1. (A) Comparison of semi-quantitative scoring for Ki67, C-Caspase-3, p62, LC3B, p21, and p27 between the NICH young age group and the NICH old age group. **(B)** Comparison of semi-quantitative scoring for Ki67, C-Caspase-3, p62, LC3B, p21, and p27 between NICH and non-proliverative vascular malformations group**. (C)** Comparison of semi-quantitative scoring for Ki67, C-Caspase-3, p62, LC3B, p21, and p27 between NICH and proliferative AVM. **(D)** Comparison of semi-quantitative scoring for Ki67, C-Caspase-3, p62, LC3B, p21, and p27 non-proliverative vascular malformations group and proliferative AVM group.

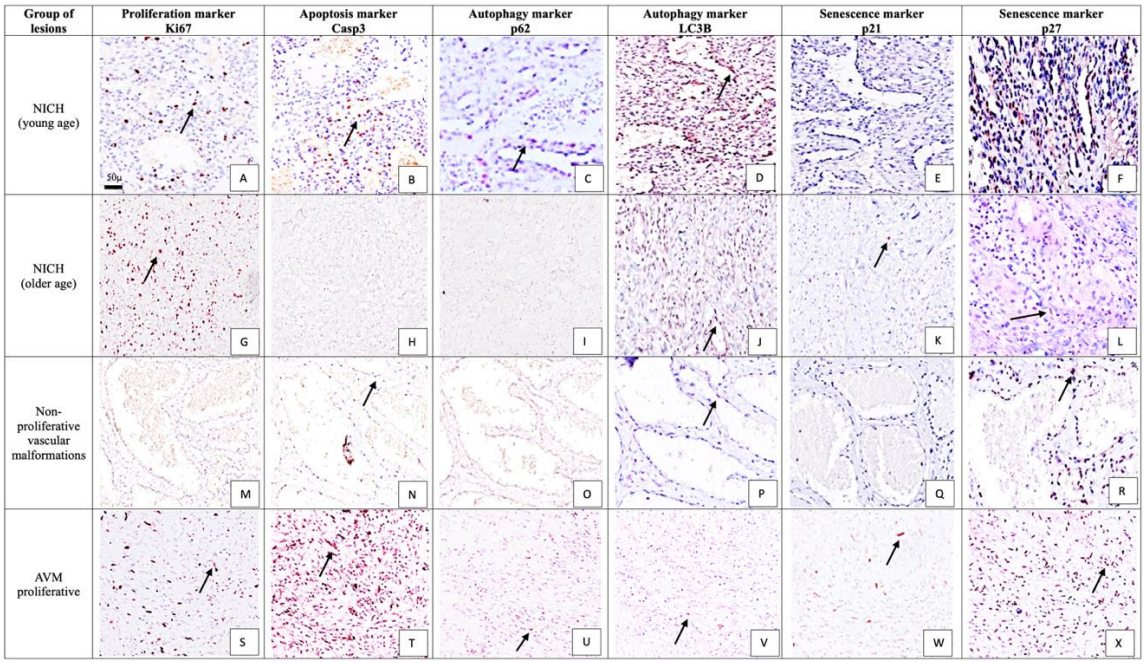

Figure 2. Immunostaining results of Ki67, Caps3, p62, LC3B, p21, and p27 in NICH young ages, NICH old ages, non-proliferative vascular malformations, and AVM proliferative lesions. Positive staining illustrates in red color using NovaRed. **(A)**Score 3 for Ki67 in NICH young age. **(B)**Score 3 C-Caspase-3 in NICH young age. **(C)**Score 1 p62 in NICH young age. **(D)**Score 3 for LC3B in NICH young age. **(E)**Score 0 for p21 in NICH young age. **(F)**Score 3 for p27 in NICH young age. **(G)**Score 3 for Ki67 in NICH old age. **(H)**Score 0 C-Caspase-3 in NICH old age. **(I)**Score 0 p62 in NICH old age. **(J)**Score 3 for LC3B in NICH old age. **(K)**Score 1 for p21 in NICH old age. **(L)**Score 3 for p27 in NICH old age. **(M)**Score 0 for Ki67 in non-proliferative vascular malformations. **(N)**Score 1 C-Caspase-3 in non-proliferative vascular malformations. **(O)**Score 0 p62 in non-proliferative vascular malformations. **(P)**Score 1 for LC3B in non-proliferative vascular malformations. **(Q)**Score 0 for p21 in non-proliferative vascular malformations. **(R)**Score 3 for p27 in non-proliferative vascular malformations. **(S)**Score 3 for Ki67 in AVM proliferative. **(T)**Score 3 C-Caspase-3 in AVM proliferative. **(U)**Score 2 p62 in AVM proliferative. **(V)**Score 2 for LC3B in AVM proliferative. **(W)**Score 2 for p21 in AVM proliferative. **(X)**Score 3 for p27 in AVM proliferative.

Co-localization with vascular smooth muscle cells

In the first 9 cases of NICH, we conducted an investigation on the cell-specific immunolocalization of p62, C-Caspase-3, and Ki67 antibodies with vascular smooth muscle cells. The results, illustrated in figure 3, showed that Ki67 was expressed within (SMA-1 positive) smooth muscle cells. Also Caspase3 activity was observed in the direct vicinity of smooth muscle cells. Furthermore, double staining of p62/SMA-1 demonstrated p62 activity primarily within the vascular smooth muscle cells. Importantly, it should be noted that specimens obtained from the old age group did not display any positive cell expression of p62.

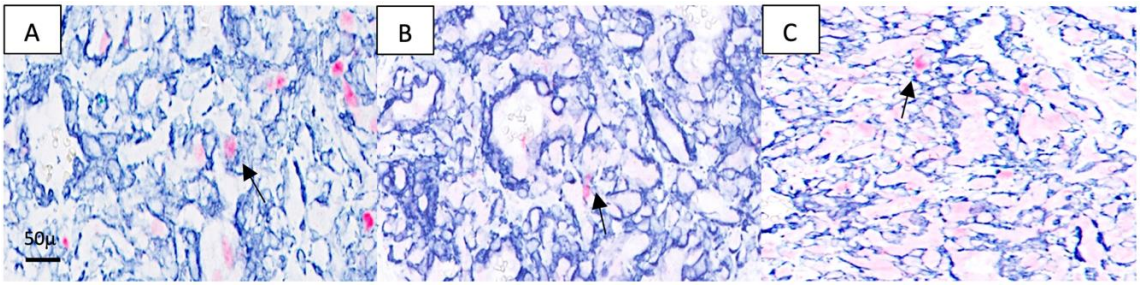

Figure 3. Double immunostaining in younger age NICH specimens of smooth muscle cells (SMA-1^{+}, stained in *blue*) combined with **(A)** Ki67, **(B)** C-Caspase-3, and **(C)** p62 (*all in red*).

4. DISCUSSIONS

The present study demonstrates distinct expression patterns of cell death and cell cycle-dependent proteins among different types of vascular malformations and NICH, and interestingly also differences among NICH patients of young and old age. The balance between cell proliferation and apoptosis is essential for tissue haemostasis, and a disbalance may contribute to tissue degeneration, developmental disorders, or cancer. This implies, that distinct patterns of vascular growth, maturation and regression could underly the differences in biological behavior, and finally differences in the pathologic outcome among these lesions. As expected on the basis of their microvascular proliferative histomorphology, both old and young NICH lesions exhibited high proliferative activity, endorsed by nuclear Ki67 expression. A noteworthy observation was the high score of apoptosis marker C-Caspase-3 in both the younger NICH group and the AVM proliferative group. In AVM, these mechanisms have not been studied yet, but the high C-Caspase-3 expression in vascular wall cells could relate to a cellular response to irregular blood flow and shear stress, potentially leading to elevated endothelial cell apoptosis and vascular remodelling(17). The significantly higher cleaved C-Caspase-3 expression in younger NICH might suggest a more active apoptotic process during the early development of NICH. This differential C-Caspase-3 expression across ages might be linked with NICH's progression commensurately to the growth of the patient, implying a more active turnover state in younger lesions. In comparison to vascular malformations, without proliferative component NICH displayed significantly higher proliferation rates, but these rates were the same as observed in arteriovenous malformations with a proliferative component. It is tempting to speculate that this diminished apoptosis rate plays a role in the lack of involution of this type of congenital hemagiomas. In this respect, it would have been of interest to compare the proliferation and apoptosis rate of NICH samples with those of the regressing types of congenital angiomas RICH and PICH. Unfortunately, given the rare occurrence of these diseases, we did not have samples available in our archive.

The situation with the expression of autophagy markers was more difficult to interpret, because both old and young NICH showed a high p62/LC3B ratio, but with almost absence of p62 expression in old lesions. The younger age group of NICH displayed significantly higher scores of vascular LC3B immunostaining, a marker for the formation of autophagosomes involved in the process of autophagy(18). This higher LC3B expression suggests an increased formation of autophagosomes in

younger NICH lesions, which could be an indication for a heightened metabolic demand or stress during early stages of lesion development. Autophagy is known to support neoplastic tumor growth and reduces their sensitivity to stimuli initiating regression(19). When compared to non-proliferative vascular malformations, both NICH and AVM proliferative lesions exhibited significantly higher LC3B scores. This finding suggests that autophagy may be more active in these vascular anomalies, potentially in response to cellular stress or to support the metabolic demands of proliferating cells(20). Inside the vascular wall, autophagy serves as an important survival pathway for EC and SMC, although this pro-survival mechanism can be overwhelmed by apoptosis such as induced by situations of high oxidative stress(21).

A second protein which serves a complementary yet distinct role in the autophagy process is p62. Expression of p62 is known to be regulated at the transcriptional level, particularly under oxidative stress. It offers a critical mechanism for protein degradation in situations of aging or environmental stress(22). In cases of NICH and AVM, an accumulation of p62 suggests a response to autophagy inhibition and oxidative stress-related transcriptional effects. Indeed, endothelial cell proliferation in AVM has been linked to reactive oxidative stress(23).

Both processes, autophagy and apoptosis, serve as critical regulators of cellular homeostasis. Autophagy can provide a survival advantage to cells under stress by recycling cellular components and providing essential nutrients. However, when the stress is excessive or prolonged, cells may switch from a survival response (autophagy) to a self-destructive one (apoptosis)(21). In this scenario, autophagy and apoptosis act as two sides of the same coin, with autophagy being a form of controlled cellular remodeling, and apoptosis serving as an ultimate self-destruction mechanism. The balance between these two processes can be particularly important in the context of proliferative lesions(24).

Our study uncovered some intriguing findings concerning the senescence markers p21 and p27. There were no differences noted in p21 expression, which was invariably low compared to p27, in NICH (old versus young) and in malformations. p21 is known to suppress cell growth by inhibiting progression to the G1 phase of cell cycle, and inhibits apoptosis(25). Previous studies have shown that an increased presence of p21 is associated with retarded cell growth following arterial damage(25), and that artificially elevating p21 levels in damaged arteries can inhibit the development of thickened artery walls. The p21 expression in NICH and proliferative AVM could be indicative of a regulatory mechanism to control the rapid growth of these malformations. As for p27, we observed its presence in all the lesions we investigated at high levels, so notably the same pattern was observed in NICH and in the proliferative type of AVM. Prior research indicates that overexpression of p27 can impede the growth of endothelial cells, and can slow down the process of new blood vessel formation(26). In a mouse model, increasing p27 slowed the recovery of blood flow and reduced the number of capillaries in the mouse's hind limb(26). The high levels of p27 we found in proliferative AVM and in both groups of NICH could suggest a mechanism striving to slow down the abnormal growth and stabilize the development of blood vessels in these lesions.

Limitations of the study. NICH are rare vascular lesions, which provided only a relatively small sample size for these investigations. Moreover, our work represents a

retrospective descriptive study on tissue markers associated with basically complex regulatory mechanisms of vessel growth homeostasis and regression in vascular anomalies. Therefore, the outcome of the study cannot be interpreted as conclusive, and we acknowledge that additional experimental research is needed to confirm our data, and to provide new avenues for further studies.

5. CONCLUSION

The present study, dealing with the in situ expression of proliferation, autophagy and senescence related markers in rare types of vascular lesions, may provide some insights into the underlying mechanisms that could contribute to the non-regressing nature of NICH and its potential relationship to vascular malformations–We revealed distinct expression patterns of cell cycle related proteins in different types of benign vascular anomalies and within the same type across different age groups. The lesion of our prime interest NICH shares certain clinical characteristics (onset in utero, no tendency to regress) with congenital vascular malformations, but displayed a persistent vasoproliferative behavior and different patterns of apoptosis, autophagy and senescence. These findings may endorse the view that NICH should not be classified as congenital vascular malformation, as has been suggested previously. However, NICH did reveal similarities with the relatively rare types of AVM with a vasoproliferative component. Therefore, it could be argued whether proliferative components of AVM are really part of the developmental disorder itself, or could represent a (reactive) epiphenomenon.

REFERENCES

1. Lee PW, Frieden IJ, Streicher JL, McCalmont T, Haggstrom AN. Characteristics of noninvoluting congenital hemangioma: A retrospective review. Journal of the American Academy of Dermatology. 2014;70(5):899–903. https://doi.org/10.1016/j.jaad.2014.01.860 PMID: 24630000

2. Nasseri E, Piram M, McCuaig CC, Kokta V, Dubois J, Powell J. Partially involuting congenital hemangiomas: A report of 8 cases and review of the literature. Journal of the American Academy of Dermatology. 2014;70(1):75–9. https://doi.org/10.1016/j.jaad.2013.09.018

3. Enjolras O, Mulliken JB, Boon LM, Wassef M, Kozakewich HPW, Burrows PE. Noninvoluting congenital hemangioma: A rare cutaneous vascular anomaly. Vol. 107, Plastic and Reconstructive Surgery. 2001. p. 1647–54. https://doi.org/10.1097/00006534-200106000-00002 PMID: 11391180

4. Larsen AK, Damsgaard TE, Hedelund L. Classification of vascular anomalies. Ugeskrift for laeger. 2018;180(36). https://doi.org/10.1177/014556130608500602 PMID: 30187855

5. Cossio ML, Dubois J, McCuaig CC, Coulombe J, Hatami A, Marcoux D, et al. Non-involuting congenital hemangiomas (NICH) with postnatal atypical growth: A

case series. Pediatric Dermatology. 2019;36(4):466–70. https://doi.org/10.1111/pde.13837 PMID: 31033005

6. Berenguer B, Mulliken JB, Enjolras O, Boon LM, Wassef M, Josset P, et al. Rapidly Involuting Congenital Hemangioma: Clinical and Histopathologic Features. Pediatric and Developmental Pathology. 2003;6(6):495–510. https://doi.org/10.1007/s10024-003-2134-6 PMID: 15018449

7. Boull C, Maguiness SM. Congenital hemangiomas. Seminars in Cutaneous Medicine and Surgery. 2016;35(3):124–7. https://doi.org/10.12788/j.sder.2016.045 PMID: 27607320

8. Wildgruber M, Sadick M, Müller-Wille R, Wohlgemuth WA. Vascular tumors in infants and adolescents. Insights into Imaging. 2019;10(1):6–8. https://doi.org/10.1186/s13244-019-0718-6

9. Mulliken JB, Enjolras O. Congenital hemangiomas and infantile hemangioma: Missing links. Journal of the American Academy of Dermatology. 2004;50(6):875–82. https://doi.org/10.1016/j.jaad.2003.10.670 PMID: 15153887

10. Meijer-Jorna LB, Van Der Loos CM, Teeling P, De Boer OJ, Florquin S, Van Der Horst CMAM, et al. Proliferation and maturation of microvessels in arteriovenous malformations - Expression patterns of angiogenic and cell cycle-dependent factors. Journal of Cutaneous Pathology. 2012;39(6):610–20. https://doi.org/c

11. Razon MJ, Kräling BM, Mulliken JB, Bischoff J. Increased apoptosis coincides with onset of involution in infantile hemangioma. Microcirculation. 1998;5(2–3):189–95. https://doi.org/10.1038/sj.mn.7300009 PMID: 9789259

12. Frischer JS, Huang J, Serur A, Kadenhe A, Yamashiro DJ, Kandel JJ. Biomolecular Markers and Involution of Hemangiomas. Journal of Pediatric Surgery. 2004;39(3):400–4. https://doi.org/10.1016/j.jpedsurg.2003.11.043 PMID: 15017560

13. North PE. Pediatric vascular tumors and malformations. Surgical Pathology Clinics. 2010;3(3):455–94. https://doi.org/10.1016/j.path.2010.07.002

14. Adams DM, Brandão LR, Peterman CM, Gupta A, Patel M, Fishman S, et al. Vascular anomaly cases for the pediatric hematologist oncologists—An interdisciplinary review. Pediatric Blood and Cancer. 2018;65(1):1–9. https://doi.org/10.1002/pbc.26716 PMID: 28727248

15. Pertiwi KR, de Boer OJ, Mackaaij C, Pabittei DR, de Winter RJ, Li X, et al. Extracellular traps derived from macrophages, mast cells, eosinophils and neutrophils are generated in a time-dependent manner during atherothrombosis. Journal of Pathology. 2019;247(4):505–12. https://doi.org/10.1002/path.5212 PMID: 30506885

16. Omori Y, Ono Y, Kobayashi T, Motoi F, Karasaki H, Mizukami Y, et al. How does intestinal-type intraductal papillary mucinous neoplasm emerge? CDX2 plays a critical role in the process of intestinal differentiation and progression. Virchows Archiv. 2020;477(1):21–31. https://doi.org/10.1007/s00428-020-02806-8 PMID: 32291497

17. Li X, Li J, Wang M, Wang J, Wang L, He H, et al. Case Report: A Rare Abdominopelvic Arteriovenous Malformation: Originating From Splenic Artery and Draining Into Portal Vein. Frontiers in Cardiovascular Medicine. 2022;9(June):1–6. https://doi.org/10.3389/fcvm.2022.916096

18. Bresciani A, Spiezia MC, Boggio R, Cariulo C, Nordheim A, Altobelli R, et al. Quantifying autophagy using novel LC3B and p62 TR-FRET assays. PLoS ONE. 2018;13(3):1–18. https://doi.org/10.1371/journal.pone.0194423 PMID: 29554128

19. Galluzzi L, Green DR. Autophagy-Independent Functions of the Autophagy Machinery. Cell. 2019;177(7):1682–99. https://doi.org/10.1016/j.cell.2019.05.026 PMID: 31199916

20. Yang ZJ, Chee CE, Huang S, Sinicrope F. Autophagy modulation for cancer therapy. Cancer biology & therapy. 2011 Jan;11(2):169–76. https://doi.org/10.4161/cbt.11.2.14663 PMID: 21263212

21. De Meyer GRY, Grootaert MOJ, Michiels CF, Kurdi A, Schrijvers DM, Martinet W. Autophagy in vascular disease. Circulation research. 2015 Jan;116(3):468–79. https://doi.org/10.1161/CIRCRESAHA.116.303804 PMID: 25634970

22. Bao J, Li G, Yuan X, Li PL, Gulbins E. Contribution of p62 to Phenotype Transition of Coronary Arterial Myocytes with Defective Autophagy. Cellular Physiology and Biochemistry. 2017;41(2):555–68. https://doi.org/10.1159/000457877 PMID: 28214847

23. Utami AM, Azahaf S, Boer OJ De, Horst CMAM Van Der, Meijer-jorna LB. A literature review of microvascular proliferation in arteriovenous malformations of skin and soft tissue. Journal of Clinical and Translation Research. 2021;7(4):540–57. https://doi.org/10.18053/jctres.07.202104.011 PMID: 34541367

24. Xi H, Wang S, Wang B, Hong X, Liu X, Li M, et al. The role of interaction between autophagy and apoptosis in tumorigenesis (Review). Oncology Reports. 2022;48(6):1–16. https://doi.org/10.3892/or.2022.8423 PMID: 36222296

25. Yang ZY, Simari RD, Perkins ND, San H, Gordon D, Nabel GJ, et al. Role of the p21 cyclin-dependent kinase inhibitor in limiting intimal cell proliferation in response to arterial injury. Proceedings of the National Academy of Sciences of the United States of America. 1996;93(15):7905–10. https://doi.org/10.1073/pnas.93.15.7905 PMID: 8755575

26. Goukassian D, DÍEz-Juan A, Asahara T, Schratzberger P, Silver M, Murayama T, et al. Overexpression of p27 Kip1 by doxycycline-regulated adenoviral vectors inhibits endothelial cell proliferation and migration and impairs angiogenesis . The FASEB Journal. 2001;15(11):1877–85. https://doi.org/10.1096/fj.01-0065com PMID: 11532967

Conflict of Interest Statement:

The author declares that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.