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Application of the 2017 criteria for vascular Ehlers-Danlos syndrome in 50 patients ascertained according to the Villefranche nosology

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Abstract

Vascular Ehlers-Danlos syndrome (vEDS) is a rare inherited connective tissue disorder due to heterozygous pathogenic *COL3A1* variants. Arterial, intestinal, and/or uterine fragility is the disease hallmark and results in reduced life expectancy. The clinical diagnosis is not always straightforward and patients' selection for molecular confirmation depends on the characteristics of applied criteria, that is, the Villefranche criteria (in use until 2017) and their revision according to the new EDS nosology. Herein, we reassessed the clinical features of 50 molecularly proven vEDS patients, diagnosed according to the Villefranche nosology between 2000 and 2016, using the 2017 classification in order to explore its clinical application. Our findings indicate that the Villefranche criteria were particularly valuable for symptomatic patients, even if with a limited specificity. Our study also suggests that the revised vEDS criteria, although expected to be more specific, might have a poorer accuracy, principally in terms of sensitivity. Both sets of criteria are less effective in presymptomatic young patients, especially in the absence of a clear-cut family history. For these patients, the careful evaluation of the cutaneous, articular, and dysmorphic features and, above all, genetic testing remain crucial to set-up proper follow-up and surveillance before catastrophic vascular and intestinal events.

KEYWORDS
COL3A1, diagnostic criteria, nosology, type III collagen, vascular Ehlers-Danlos syndrome

1 | INTRODUCTION

Vascular Ehlers-Danlos syndrome (vEDS) is a rare autosomal dominant connective tissue disorder with an estimated prevalence of 1/50.000-150.000. It results from pathogenetic variants in *COL3A1*, encoding type III collagen that is the major expressed collagen in blood vessels and hollow organs. Severe tissue fragility is the leading feature and may present with arterial aneurysm, arterial dissection and rupture mainly of the middle-sized arteries, spontaneous bowel perforation, and uterine rupture during pregnancy.[1,2] Life span of affected individuals is reduced to a median age of 50 years and the major cause of death is arterial dissection or rupture with organ failure. In the absence of a family history, vEDS is usually suspected in the context of a major complication or post-mortem.[1-5] Therefore, early recognizing the disorder is of utmost importance for preventing and early treating potentially lethal complications, but the clinical diagnosis of apparently sporadic asymptomatic patients might be challenging. Since 1998, vEDS diagnosis was based on the criteria of the Villefranche nosology.[6] In 2017, the International Classification of EDS identified a novel set of criteria for selecting patients for confirmatory

molecular testing.[7] Based on the Villefranche nosology, vEDS was suspected in the presence of at least two major criteria, whereas minor criteria were supportive (Table 1). In the 2017 EDS classification, the major and minor criteria were changed (Table 1) and the following minimal criteria, which are intended for prompting molecular testing, were introduced: (a) positive family history of the disorder, (b) arterial rupture or dissection in individuals less than 40 years of age, (c) unexplained sigmoid colon rupture, (d) or spontaneous pneumothorax in the presence of other features consistent with vEDS. Testing for vEDS should also be considered in patients presenting with a combination of minor clinical criteria.[7] Moreover, very recently, the vEDS GeneReview suggested molecular testing also in patients with any one of the new major criteria.[2]

TABLE 1 Diagnostic criteria for vEDS according to the Villefranche nosology and the 2017 International Classification of EDS

Villefranche nosology[6]	
Major criteria	Minor criteria
• Thin, translucent skin	• Acrogeria
• Arterial/intestinal/uterine fragility or rupture	• Hypermobility of small joints
• Extensive bruising	• Tendon and muscle rupture
• Characteristic facial appearance	• Talipes equinovarus
	• Early-onset varicose veins
	• Arteriovenous, carotid-cavernous sinus fistula
	• Pneumo(hemo)thorax
	• Gingival recession/fragility
	• Positive family history, sudden death in (a) close relative(s)
2017 International Classification of EDS 7	
Major Criteria	*Minor Criteria*
• Family history of vEDS, with documented causative *COL3A1* variant • Arterial rupture at a young age • Spontaneous sigmoid colon perforation in the absence of known diverticular disease or another bowel pathology • Uterine rupture during the third trimester in the absence of previous cesarean section and/or severe peripartum perineum tears • Spontaneous carotid-cavernous sinus fistula (sCCF) formation	• Bruising unrelated to identified trauma and/or in unusual sites such as cheeks and back • Thin, translucent skin with increased venous visibility • Characteristic facial appearance • Spontaneous pneumothorax • Acrogeria • Talipes equinovarus • Congenital hip dislocation • Hypermobility of small joints • Tendon and muscle rupture • Keratoconus • Gingival recession/fragility • Early onset varicose veins (under age 30 and nulliparous if female)

Abbreviation: vEDS, Vascular Ehlers-Danlos syndrome.

Because of clinical overlap with other EDS types, some forms of Loeys-Dietz syndrome, Marfan syndrome, arterial tortuosity syndrome, and familial arterial aneurysm and dissection syndromes, the diagnosis of vEDS must be always confirmed by identification of a pathogenic *COL3A1* variant.[1,2,7]

A recent work evaluated the accuracy of the diagnostic criteria of both nosologies in terms of sensitivity (Se), and specificity (Sp), according to the results of genetic testing.[8] The study cohort consisted of 519 patients, who underwent genetic testing that identified a *COL3A1* variant in 31.8% of the cases. The Villefranche criteria were met for 47.7% of patients with a Se of 79% and a Sp of 67%. The diagnostic accuracy was highest for symptomatic probands that showed a better Se (92%), which was lower for young patients (≤ 25 years). The authors provided evidence that the revised nosology seems to be less accurate than the Villefranche classification, mainly due to lack of Se. Hence, they argued that the Villefranche nosology should be preferred in assessing vEDS patients in specialized clinics.

Herein, we re-evaluated a cohort of 50 molecularly confirmed vEDS patients originally ascertained according to the Villefranche nosology. We determined the distribution of major, minor, and minimal criteria of the 2017 nosology in order to investigate their application in the real world.

2 | PATIENTS AND METHODS

Thirty-seven index-patients and 13 relatives with vEDS were evaluated from 2000 to 2016 in the specialized outpatient clinics for the diagnosis of EDS, that is, the Ehlers-Danlos Syndrome and Inherited Connective Tissue Disorders Clinic (CESED) at the University Hospital Spedali Civili of Brescia. All clinical signs included in the historical Villefranche nosology[6] were evaluated prior to genetic testing. When possible, the presence of arterial aneurysms was investigated by cerebral, thoracic, abdominal MRI, and heart ultrasound. Physical examination of available patients consisted in assessment of facial dysmorphism, external ocular features, joint mobility, and skin manifestations. Skin was touched and evaluated for its texture/consistence. Smooth soft/velvety/doughy skin was an entirely subjective feeling developed during clinical practice. Inspection of skin consistency was performed by touching different sites including arms, thorax, hands, and legs. Skin hyperextensibility was qualitatively evaluated as previously described for classical EDS.[9] A set of objective facial and mucocutaneous features was also selected. Ocular signs included keratoconus, epicanthal folds, upper palpebral ptosis, hypo/hypertelorism, and elongated/up-slanting palpebral fissures. Additional investigated facial dysmorphisms included micro/retrognathia, low-set ears, elongated philtrum, and hypoplastic auricular lobe. Further mucocutaneous features comprised livedo reticularis, keratosis pilaris/hyperkeratosis of extensor surfaces, striae distensae, skin wrinkling/redundancy, acquired cutis laxa/acrogeria, keloid formation and elastosis perforans serpiginosa, umbilical/incisional/inguinal hernia, piezogenic papules, uvular abnormalities, and bluish sclerae. In patients with extensive easy bruising, routine coagulation tests were

performed with normal results in all. Joint hypermobility (JHM) was evaluated according to the Beighton score (BS).[6] In adults with a BS <5, the presence of historical JHM was routinely investigated with the five-point questionnaire.[10] Other evaluated musculoskeletal features included sprains, temporomandibular joint dysfunction, chronic pain, scoliosis/kyphoscoliosis, cubita/genua valga, hallux valgus, and pes planus.

Molecular diagnosis was achieved in compliance with the Italian legislation on genetic diagnostic tests by bidirectional Sanger sequencing of the *COL3A1* coding sequences and exon-intron junctions with the BigDye Terminator v1.1 Cycle Sequencing kit on an ABI 3130XL Genetic Analyzer according to manufacturer's protocols (Applied Biosystems) and by MLPA analysis with the SALSA MLPA Probemix P155 according to manufacturers instructions (MRC-Holland).

After the publication of the 2017 EDS classification, patients' clinical features were reanalyzed according to the new criteria. Assessment between the presence/absence of investigated features and selected dichotomous variables, that is, age at examination ≤ or >18 years and gender, was performed with the chi-square test with Yates's correction or Fisher's exact test whenever the count was insufficient. Analysis was carried out with the GraphPad Software and considering significant *P*-values when less than .05.

This study was carried out from routine diagnostic activity and does not request formal ethics review. The study follows the principles outlined in the Helsinki Declaration and patients gave written informed consent for publication of their clinical and molecular data.

3 | RESULTS

3.1 | General and molecular findings

Among the 50 vEDS patients from 37 families, 32 were females (64%), and 18 were males (36%) (sex ratio: 1.78). By considering the index-cases, the sex ratio was 1.85 (24 females and 13 males). Twenty-two index-patients were previously published in Reference 11, whereas 18 from 15 additional families were novel. Ten additional affected family members from eight pedigrees described in Reference 11 were also reported.

In all new families, a *COL3A1* pathogenetic variant was identified and submitted to the LOVD EDS Variant Database (http://www.le.ac.uk/ge/collagen/). In particular, seven variants were known (four glycine substitutions and three in-frame exon skipping splice variants) and eight were novel (five glycine substitutions, one in-frame exon skipping splice variant, and one multi-exon deletion detected by MLPA, all within the collagenous domain of the protein with a predicted dominant negative effect, and one small deletion/insertion likely representing a null-allele). Table 2 summarizes the pathogenic variants identified in the 37 families.

TABLE 2 Pathogenic *COL3A1* variants identified in our patients' cohort

Family	LOVD ID	DNA change	Mutation effect	Family	LOVD ID	DNA change	Mutation effect
1	P.18[a]	c.3417+1G>A[b]	Skipping of exon 47	20	P.8 [a]	c.1761+1G>A[b]	Skipping of exon 25
2	AN_002577	**c.2303G>T**	**p.(Gly768Val)**	21	P.15 [a]	c.2797G>A	p.(Gly933Arg)
3	P.16[a]	c.2897G>T[b]	p.(Gly966Val)	22	AN_002584	c.3229G>A	p.(Gly1077Ser)
4	AN_002578	**c.1466G>T**	**p.(Gly489Val)**	23	P.19[a]	c.3508G>A[b]	p.(Gly1170Ser)
5	P.10[a]	c.1835G>A[b]	p.(Gly612Asp)	24	AN_002585	c.2931+1G>A[b]	Skipping of exon 41
6	P.17[a]	c.2986G>A	p.(Gly996Arg)	25	P.6[a]	c.1474G>A	p.(Gly492Arg)
7	P.4[a]	c.951+6T>C[b]	Skipping of exon 14	26	P.20[a]	c.3509G>A[b]	p.(Gly1170Asp)
8	AN_002579	**c.2337+2T>A**	**Skipping of exon 34** (Pr)	27	P.14[a]	c.2735G>C	p.(Gly912Ala)
9	AN_002580	c.1869+5G>A[b]	Skipping of exon 27	28	AN_002586	c.583G>C[b]	p.(Gly195Arg)
10	P.12[a]	c.2194G>A[b]	p.(Gly732Arg)	29	AN_002587	**c.1349G>T**	**p.(Gly450Val)**
11	P.7[a]	c.1655G>A[b]	p.(Gly552Glu)	30	AN_002590	c.2896G>T[b]	p.(Gly966Cys)
12	P.11[a]	c.2077G>A	p.(Gly693Arg)	31	P.1[a]	c.674G>A	p.(Gly225Asp)
13	AN_002581	**c.(744+1_799−1)_(996+1_1150-1)del**	**Multi-exon deletion**	32	P.2[a]	c.709G>A[b]	p.(Gly237Arg)
14	P.3[a]	c.836G>A[b]	p.(Gly279Asp)	33	AN_002591	**c.750delCinsAA**	**p.(Arg252Glyfs* 24)**
15	P.5[a]	c.1347+1G>A[b]	Complex splice outcome	34	P.21[a]	c.3509G>A[b]	p.(Gly1170Asp)
16	AN_002582	**c.2060G>T**	**p.(Gly687Val)**	35	AN_002593	c.1662+1G>C[b]	Skipping of exon 24
17	P.22[a]	c.3563G>T	p.(Gly1188Val)	36	AN_002594	**c.620G>C**	**p.(Gly207Ala)**
18	P.9	c.1808G>A[b]	p.(Gly603Asp)	37	P.13	c.2607+42607+7del	Skipping of exon 38
19	AN_002583	c.638G>A[b]	p.(Gly213Asp)	***COL3A1* ref. seq: NM_000090.3, NP_000081.1**			

Abbreviation: Pr: in silico prediction.

[a]Published in Drera et al. 2011[11]; novel variants not previously reported in LOVD (last access, 11/06/2019) are in bold.

[b]Recurrent mutation (reported at least twice in LOVD).

Of 37 index-cases 21 were sporadic with a verified de novo mutational event. Twenty patients from 10 different families were clinically and molecularly diagnosed at the same time, whereas 3 affected relatives from 2 previously described pedigrees[11] were evaluated after the diagnosis of the index-patient. In addition, in 8 of these 10 families, pedigree reconstruction indicated the presence of further presumably affected relatives, who died of a vascular or visceral event before ascertainment. In the six remaining index-cases, a positive family history was assumed as well, since they reported sudden death for vascular and/or intestinal rupture in a close/first-degree relative. Detailed clinical and molecular features of patients are reported in Supporting Information, Table S1. Frequencies of selected features and their combinations are reported in Figure 1, Table 3, and Tables S2-S6.

Age at examination ranged from 3 to 68 years (mean 31.1; SD [SD] 14.2). In particular, age range was 3 to 68 years (mean 33.34; SD 14.2) for females, and 3 to 43 years (mean 27.1; SD 13.7) for males. Patients aged 18 years or less were 11 (22%; including four females and seven males; eight were children and three adolescents), 39 patients (78%) were adults (>18 years). Living patients are 76% (38/50), while 24% of the patients were deceased at the time of writing (12/50, mean age at death 29.1 years; SD 11.3; range 13-43). Deceased patients were more commonly males (8/18, 44.5%) than females (12.5%, 4/32 *P*-value .0283). Death occurred earlier in males (mean age of death 27.13 years; SD 12.4; range 13-42) than in females (mean age of death 33.0; SD 8.68; range 22-43), although not statistically significant. Three out of the eight deceased males were

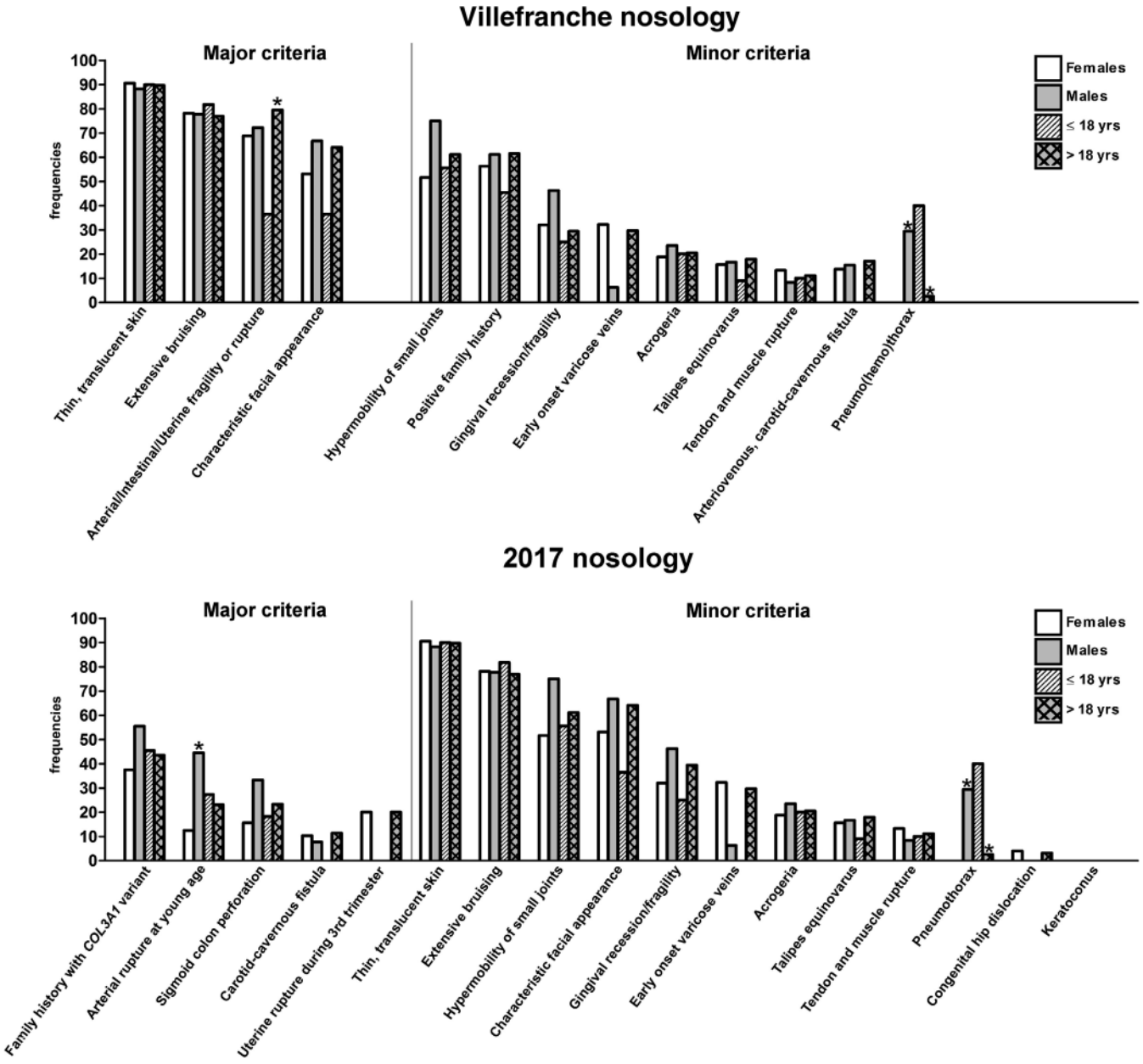

FIGURE 1 Frequencies of major and minor diagnostic criteria of the Villefranche nosology and of the 2017 EDS classification in our cohort of 50 molecularly proven vEDS patients

TABLE 3 Frequencies of the minimal criteria suggestive for vEDS of the 2017 EDS nosology by sex and age

Criteria (%)	Total	Females	Males	*P*-value[A]	Patients ≤18	Patients >18	*P*-value[B]
Family history of the disorder	29/50 (58.0)	18/32 (56.3)	11/18 (61.1)	.971	5/11 (45.0)	24/39 (61.5)	.543
Arterial rupture or dissection in individuals < 40 years	20/50 (40.0)	12/32 (37.5)	8/18 (44.4)	.856	3/11 (27.2)	16/39 (41.0)	.632
Unexplained sigmoid colon rupture	11/50 (22.0)	5/32 (15.6)	6/18 (33.3)	.273	2/11 (18.2)	9/39 (23.1)	.947
Spontaneous pneumothorax in the presence of other features consistent with vEDS	5/48 (10.4)	0/31 (0.0)	5/17 (29.4)	**.004**	4/10 (40.0)	1/38 (2.6)	**.004**

Note: Significant *P*-values <.05 are in bold. A: *P*-values females vs males; B: *P*-values patients ≤18 years vs patients >18 years.
Abbreviation: vEDS, Vascular Ehlers-Danlos syndrome.

aged less than 18 years. For two patients, a 16-year-old male and a 35-year-old female, vEDS suspicion was made after death, which occurred due to an axillary artery and aortic rupture, respectively. The other patients died after vEDS diagnosis for a major vascular (four for aortic dissection/rupture, and one for fatal hemorrhage in spontaneous pneumothorax) or visceral (four for sigmoid colon perforation, and one for splenic rupture) event. Major vascular complications (20 of 37 index-patients, 54%) and visceral ruptures (11/37, 30%) were also the main reasons leading to clinical ascertainment and/or genetic testing, except for six probands (16%) who were referred for unusual physical findings, that is, thin, translucent skin, extensive bruising, and characteristic facial appearance.

3.1.1 | Major Villefranche criteria

Concerning the major Villefranche criteria, 89.8% of patients had thin/translucent skin, 78% extensive bruising with hematomas and ecchymoses, 70% arterial/intestinal/uterine fragility, and 58% a peculiar facial appearance, that is, thin, delicate, and pinched nose, thin lips, tight skin, hollow cheeks and prominent staring eyes, and tight, firm, lobeless ears. All these features did not show any correlation with sex and age, except for arterial/intestinal/uterine fragility that was significantly higher in adults than in young individuals (Figure 1, Table S2). Ninety-four percent of patients (47/50) and 97.3% (36/37) of index-cases, respectively, showed at least two major criteria of the Villefranche nosology necessary for a vEDS suspicion prompting molecular confirmation (Table S3). A 21-year-old female presented one major criterion only (ie, sigmoid colon perforation) and two minor criteria, that is, hypermobility of small joints and positive family history (Tables S1 and S3). The two remaining patients not meeting the Villefranche criteria were family relatives of different probands who experienced vascular complications, namely a 3-year-old female without any sign indicative of vEDS and a 11-year-old male presenting thin, translucent skin, and pneumothorax.

In the entire cohort, thin, translucent skin plus extensive bruising was the most frequent combination (71.4%), followed by thin, translucent skin plus arterial/intestinal/uterine fragility (59.2%), arterial/intestinal/uterine fragility plus extensive bruising (56%), and thin, translucent skin plus peculiar facial appearance (53.1%); the other combinations were observed with an occurrence lower than 50%. The percentage of patients who presented the simultaneous presence of 3 major criteria ranged from 34% (arterial/intestinal/uterine fragility or rupture plus extensive bruising plus peculiar facial appearance) to 46.9% (thin/translucent skin plus arterial/intestinal/uterine fragility or rupture plus extensive bruising). Finally, 28.6% of the patients showed the presence of all the major criteria. All these combinations did not show any significant difference between sex and age (Table S4).

Regarding arterial/intestinal/uterine fragility, 28 of 50 patients of our cohort (56%, 18 adult females, 7 adult, and 3 young males) suffered from at least one vascular event and 71.4% of them (20/28) showed multiple vascular complaints (Table S1). The mean age of the first vascular complication was 33.6 years (SD 12.1, range 13-76). The most frequent vascular manifestations were arterial dissection/rupture (25/28), followed by aneurysmal dilatations (19/28), spontaneous carotid-cavernous sinus fistula (sCCF) (4/28), and other arteriovenous fistulae (2/28). Arterial dissection was more frequently observed at iliac, aortic, and carotid arteries, followed by mesenteric, renal, hepatic, vertebral, celiac, and splenic arteries. Rupture occurred at several sites, in particular, splenic, hepatic, and aortic arteries were mostly affected, whereas (epi)gastric, humeral, axillary, and carotid ruptures were less frequently observed. Similarly, aneurysms occurred at different locations including the visceral branches of the aorta (especially splenic, celiac, renal, hepatic, and iliac arteries) and the cerebral, carotid, and vertebral arteries. In seven patients, arterial rupture occurred in an otherwise normal vessel as shown by MRI. Four adult patients (three females and one male) suffered of hemorrhagic stroke.

Intestinal fragility (ie, sigmoid colon perforation) was observed in 11 of 50 patients (22%, 5 females, 6 males; mean age 28.82; SD 10.65; range 11-46). Five patients experienced multiple colon perforations and in two of them leakage from the anastomosis of previous segmental resections was observed. Eight patients (16%) presented with a combination of both vascular and intestinal events. Other visceral events encountered in our cohort included ruptures of spleen, pyosalpinx, gallbladder, vagina, and liver. A 21-year-old female patient experienced esophageal rupture during vomiting. Ten out of 32 women had at least one pregnancy and nine of them had multiple pregnancies. Seven of the surviving children were affected. Single and multiple miscarriages occurred in three and one patients, respectively. Uterine rupture in the last trimester of pregnancy occurred in two patients and led to fetal demise in both. Uterine hemorrhage and a fourth-degree vaginal laceration during delivery happened in four patients, whereas three women had uncomplicated pregnancies and

delivery. Premature rupture of membranes and consequent preterm delivery occurred in four patients all with an affected fetus (Table S1).

3.1.2 | Minor Villefranche criteria

Concerning the minor Villefranche criteria, hypermobility of small joints (60%) and a positive family history of vEDS (58%) were the most frequent features, followed by gingival recession/fragility (36.6%), early onset varicose veins (23.4%), and acrogeria of the hands and/or feet, that is, very thin dorsal skin with atrophic fine wrinkling (20.4%), talipes equinovarus (16%), sCCF and other arterio-venous fistulae (14.3%), tendon and muscle rupture (10.9%), and spontaneous pneumo(hemo)thorax (10.4%). All these minor criteria did not show any correlation with sex and age, except for pneumo(hemo)thorax that was observed only in five males, four of them were aged ≤18 years (Figure 1, Tables S1 and S2). Among these males, a fatal hemorrhage in spontaneous pneumothorax occurred in an 18-year-old man during outdoor activity in the mountains and a 16-year-old patient, after a shoulder distractive trauma during a volleyball match, experienced a massive left hemothorax associated with lung collapse and laceration of the ipsilateral axillary artery leading to lethal hemorrhagic shock.

3.1.3 | Additional features not included in the Villefranche nosology

Unlike in other EDS forms, skin, apart from being thin and translucent in most cases (89.8%), was always inelastic, except for three patients who showed mild skin hyperextensibility. Poor quality wound healing was observed in 22 of 48 patients (45.8%) leading to aberrant scarring with small, atrophic cigarette paper scars. Striae distensae were observed in 10 of 48 patients (20.8%), inguinal hernias requiring surgery in 5 of 38 (13.1%), and light blue sclerae in 8 of 17 (47%). Keloid formation and elastosis perforans serpiginosa were not observed in our cohort (Table S1).

Although hypermobility of small joints was the most frequent minor criterion, generalized JHM according to the BS was present only in 26% of the patients (12/45 BS ≥5, 7 adult females, 3 young, and 2 adult males); complications of JHM, that is,., (sub)luxations (especially at jaw, shoulders, ankle, and knees) and/or sprains, were present in all of these patients as well as in seven patients with BS <5 referring historical hypermobility according to the five-point questionnaire.[10] Chronic pain was referred in 13 of 34 (38.2%) patients. Other musculoskeletal features included scoliosis/kyphoscoliosis (15/26, 57.6%), temporomandibular joint dysfunction (11/26, 42.3%), minor body asymmetries (11/28, 39.2%), cubita, genua, and hallux valgus (9/24, 37.5%), and pes planus (13/36, 36.1%) (Table S1).

3.2 | Application of the revised criteria according to the 2017 International Classification of EDS

Overall, the five new major criteria were all less frequently observed than those of the Villefranche nosology. In particular, a family history of vEDS with a documented *COL3A1* pathogenic variant was present in 44% of the patients, arterial rupture at young age in 24% with a statistically significant higher incidence in males than in females (44.4% vs 12.5%, *P*-value .028), spontaneous sigmoid colon in 22%, sCCF formation in 9.5%, and uterine rupture during the third trimester in 20% of the females who experienced pregnancies (Figure 1 and Table S5). In the entire cohort, 70% of patients (35/50) showed at least one major criterion, whereas by considering index-patients only, this percentage decreased to 55% (20/37), given the high frequency of family history of vEDS with a documented pathogenic variant. Indeed, none of our index-cases was referred for evaluation with a positive *COL3A1* test.

Thin, translucent skin (89.8%), extensive bruising (78%), and characteristic facial appearance (58%), which previously were three of the four major Villefranche criteria, have become the most common minor criteria of the 2017 nosology, together with hypermobility of small joints (60%) and gingival recession/fragility (36.6%), followed by the others, all with a frequency lower than 25%. The two newly introduced minor criteria, that is, congenital hip dislocation and keratoconus, were uncommon, since the former was recognized in 1 patient out of 41 (2.4%) and the latter was absent in 24 investigated patients (Figure 1 and Tables S1 and S5).

The 2017 nosology also defined minimal criteria that may rise clinical suspicion of vEDS. Ideally, meeting one or more of these minimal criteria should prompt molecular testing. In our cohort, a positive family history of vEDS was observed in 58% (29/50) of the patients, arterial rupture or dissection before the age of 40 in 40% (20/50), unexplained sigmoid colon rupture in 22% (11/50), and spontaneous pneumothorax in the presence of other somatic features of vEDS in 10.4% (5/48) (Table 3). Overall, 84% of the patients (42/50) showed at least one minimal criterion, whereas by considering index-patients, this percentage was 78.3% (29/37). Of note, the three individuals mentioned above who did not meet the Villefranche criteria, satisfied the minimal criteria of the 2017 nosology, since a patient suffered from sigmoid colon perforation, another displayed spontaneous pneumothorax together with other features of vEDS, and all presented positive family history (Table S3).

Among the eight female patients who did not present one or more minimal 2017 criteria, two adults (>40 years) showed sCCF (a new major criterion) together with thin, translucent skin, extensive bruising, and peculiar facial appearance. The remaining six patients (mean age 29.8 years, range 7-42) showed a combination of minor criteria ranging from 6 to 3 and the combination of thin, translucent skin plus extensive bruising was present in all (Table S3). The other minor criteria included hypermobility of small joints, which was observed in four patients, and peculiar facial appearance, gingival recession/fragility, talipes equinovarus, early onset varicose veins, and acrogeria, which were all detected in two patients. Consistently, in the entire cohort, the combinations of minor criteria with an occurrence rate above 50% are those joining thin, translucent skin, extensive bruising, and peculiar facial appearance (Table S6).

Taken together, merging the minimal criteria of the revised EDS nosology[7] and the recommendation of the updated vEDS GeneReview,[2] all patients of our cohort would have been eligible for

genetic testing, since they presented either one or more major (70%) or at least one minimal (84%) or a combination of minor criteria (100%).

4 | DISCUSSION

This work presents data on diagnostic criteria and some additional features in 50 vEDS patients that was carried out in a clinical setting by using the Villefranche nosology[6] as the unique tool for clinical assessment and molecular testing. Our findings highlight that the presence of two or more major Villefranche criteria were a truthful indication for a suspicion of vEDS. Indeed, 94% of patients fulfilled the Villefranche nosology and only three patients did not show two major criteria.

Nine additional probands with vascular events and additional suggestive features of vEDS were also referred to our center. All resulted negative for the presence of a *COL3A1* causal variant, although meeting the Villefranche criteria. Among them, six were afterwards molecularly diagnosed as Loeys-Dietz syndrome (two reported in References 12, 13 and three unpublished patients), and one as arterial tortuosity syndrome.[14] This indicates that the Villefranche criteria are predictive for a positive *COL3A1* molecular testing but are not fully specific for vEDS. Molecular testing, extended to the major molecular differential diagnoses in case of negative results, is always indicated for diagnosis confirmation and optimal clinical management.[15-17]

The recent study of Henneton et al[8] calculated for the Villefranche criteria an overall diagnostic odds ratio score (DOR) of 7.8%, mainly due to the limited specificity (Sp) of arterial fragility. Indeed, among the 384 index-patients, who were tested not only in the presence of two or more major diagnostic criteria (209 patients, 54.4%) but also in the context of arterial fragility alone (175 patients, 45.5%), only 105 patients (27.3%) were found to harbor a pathogenic variant. By comparing *COL3A1* positive patients with those without a pathogenic variant, the frequencies of all major criteria were statistically higher in the mutation positive cohort, except arterial/intestinal/uterine fragility or rupture that was observed with similar rates in both groups. Concerning minor criteria, positive family history, acrogeria, early-onset varicose veins, pneumo(hemo)thorax, talipes equinovarus, tendon and muscle rupture, and arteriovenous fistula and/or sCCF were all statistically more frequent in the *COL3A1* positive patients, whereas hypermobility of small joints and gingival recession/fragility showed similar rates. Although we did not compare mutation positive and negative patients, the rates of arterial/intestinal/uterine fragility or rupture, thin, translucent skin, extensive bruising, peculiar facial appearance, positive family history, early-onset varicose veins, talipes equinovarus, and pneumo(hemo)thorax observed in our study are comparable with those of the *COL3A1* positive cohort reported by Henneton et al.[8] Differences in sample size, ethnicity or patients' selection bias or subjective interpretation of criteria without a precise formal definition might explain the higher rate of hypermobility of small joints, gingival recession/fragility, arteriovenous, carotid-cavernous sinus fistula, and tendon and muscle rupture, and the lower rate of acrogeria in our sample.

Henneton et alalso calculated the accuracy of each individual Villefranche criterion for probands. Three out of the four major criteria showed acceptable DOR scores, that is, characteristic facial appearance (DOR 14.3, sensitivity (Se) 70%, Sp 86%), extensive bruising (DOR 6.58, Se 67%, Sp 77%), and thin, translucent skin (DOR 5.78, Se 73%, Sp 68%), whereas arterial/intestinal/uterine fragility or rupture showed a DOR score of 1.64 (Se 84%, Sp 24%). After dissecting the different organ fragilities, only intestinal rupture displayed an adequate DOR score (10.9) with high Sp (97%) but low Se (27%). Consistently with these results, our unique proband who did not show two major criteria according to the Villefranche nosology underwent genetic testing for the presence of sigmoid colon rupture.

Still in Henneton and coauthors, all minor criteria, except hypermobility of small joints (Sp 59%), showed high Sp with a limited Se. A Sp of 99% was obtained either for arteriovenous fistula and/or sCCF (Se 6%), clubfoot (Se 12%), or tendon and muscle rupture (Se 7%). The other minor criteria ranged from a Sp 96% for pneumo(hemo)thorax (Se 14%) to 81% for positive family history (DOR 2.96). Se scores ranged from 53% for acrogeria (Sp 92%) to 6% for arteriovenous fistula and/or sCCF. Of note, in the new nosology, sCCF is considered a major criterion. In our cohort, sCCF was found in 9.5% of patients, in line with a recent study reporting a similar frequency (9.8%) in a cohort of 133 molecularly proven vEDS patients,[18] emphasizing that sCCF, although rare, is highly characteristic of vEDS.

Spontaneous pneumo(hemo)thorax was recently highlighted as an early manifestation of vEDS by frequently preceding a major arterial/intestinal event.[19] Accordingly, the 2017 classification identified pneumothorax plus other suggestive vEDS features as a minimal criterion.[7] In our cohort, spontaneous pneumothorax was observed in five males only. In two of them, pneumothorax was associated with a lethal vascular event and in one it occurred 1 year before a fatal intestinal perforation. Notably, spontaneous pneumothorax (together with thin, translucent skin, and family history) was present in one of the three relatives not meeting the Villefranche criteria but respecting the new minimal criteria for molecular testing.

Henneton et al[8] also assessed the accuracy of the revised criteria, showing that they were less precise than the Villefranche nosology. In particular, the authors disclosed for probands lower Se (68% vs 92%) and DOR score (4.04 vs 18.1). Since the new major criteria are more stringent, the Sp was slightly higher, particularly for index-patients ≥40 years old (94% vs 58%). Young patients seemed less fit for accurate vEDS detection (DOR 2.36), as observed for the Villefranche nosology. The low frequencies of the revised major criteria identified in our cohort, except family history of vEDS with a documented *COL3A1* pathogenic variant (44%), corroborate these findings and highlight that the early diagnosis of asymptomatic and (apparently) sporadic cases remains difficult. Considering that the new nosology does not provide a cut-off number of criteria for genetic testing and that evocative clinical criteria have been incremented, with a clear intention of broadening clinical/molecular screening of likely affected individuals, in our opinion, the 2017 nosology is more than

appropriate when applied in its broadest sense. Molecular testing for vEDS in the presence of any one of the major, minimal, or at least two minor criteria should allow high diagnostic yields. Based on the specific clinical presentation, the molecular approach might be different, ranging from single-gene sequencing integrated by MLPA for highly suggestive phenotypes to NGS, including not only multigene panels but also clinical exome, for cases without a clear-cut phenotype.

The overall results of this study suffer of the limits related to the samples' size and the risk of selection bias. In fact, as a tertiary center for the diagnosis of EDS, our patients are highly selected, and this might affect the rate estimates of specific diagnostic criteria in this sample.

In conclusion, here we explored retrospectively the diagnostic accuracy of the 2017 classification in suspecting vEDS in a patients' cohort with causative variants in *COL3A1* and previously ascertained by the Villefranche nosology. Our findings further define the diagnostic weight of all clinical signs associated to vEDS that should contribute to the rapid ascertainment of the prospective vEDS patient according to the 2017 EDS nosology. This study corroborates the awareness that early diagnosis of young patients without overt personal or familial phenotypic marks of the disease remains challenging. For these patients, the evaluation of the minor cutaneous, articular, and dysmorphic features and, above all, genetic testing are expected to increase the diagnostic rate. Prompt recognition of the condition, appropriate referral to a care team that should include the medical geneticist, general surgeon, vascular surgeon, cardiologist, and physician will all assist in reducing the potentially life-threatening complications of this severe condition.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

DATA ACCESSIBILITY

All data generated or analyzed for this study are included in the article and its supplemental files.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of this article.

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