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ORIGINAL ARTICLE

Accuracy of Clinical Diagnostic Criteria for Patients With Vascular Ehlers-Danlos Syndrome in a Tertiary Referral Centre

BACKGROUND: Vascular Ehlers-Danlos syndrome is a rare inherited connective tissue disease secondary to mutations within the COL3A1 gene. The diagnosis of vascular Ehlers-Danlos syndrome is challenging, and patient selection for genetic testing relies on diagnostic criteria, which have never been evaluated.

METHODS: All patients seen at a dedicated tertiary referral center for a suspicion of vascular Ehlers-Danlos syndrome between January 2001 and March 2016 were retrospectively included in a diagnostic accuracy study. Major and minor diagnostic criteria of the Villefranche classification were tested for sensitivity, specificity, positive and negative predictive values, according to results of genetic testing.

RESULTS: N=519 patients were eligible for analysis dividing into n=384 probands and n=135 relatives. A pathogenic COL3A1 variant was identified in n=165 (31.8%) patients. The Villefranche criteria were met for n=248 patients with a sensitivity of 79% (95% CI, 0.72–0.85) and a negative predictive value of 87% (95% CI, 0.83–0.91). Diagnostic accuracy was highest for symptomatic probands (sensitivity 92%; negative predictive value 95%) with limited specificity (60%). Probands ≤25 years had the worst diagnostic performance. The revised diagnostic Criteria (2017) were less accurate than the Villefranche classification (overall diagnostic odds-ratio, 4.17 versus 7.8; probands diagnostic odds-ratio, 4.04 versus 18.1; and probands ≤25 years diagnostic odds-ratio, 2.36 versus 5.1) mainly due to a lack of sensitivity.

CONCLUSIONS: The Villefranche criteria provide accurate detection of symptomatic probands in specialized practice but have limited specificity. The revised diagnostic criteria for vascular Ehlers-Danlos syndrome have increased specificity, but its overall performance is poorer. The early clinical diagnosis of probands without family history is not addressed by both diagnostic classifications.

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Ehlers-Danlos Syndromes (EDS) are a heterogeneous group of inherited connective tissue disorders characterized by hyperlaxity, skin hyperextensibility, and tissue fragility.[1,2] In 1997, the Villefranche classification described 6 major subtypes.[3] Vascular EDS (vEDS; OMIM No. 13050) is the most severe form, characterized by the occurrence in the early adulthood of spontaneous arterial ruptures or dissections, gastrointestinal perforations, or other organ fragility.[4–6] It is a rare, dominantly inherited disorder secondary to pathogenic variants in the *COL3A1* gene encoding for the pro-α1 chain of type III procollagen.[7,8] Formal early diagnosis of vEDS is critical to ensure a dedicated medical, interventional, andr surgical management in case and in prevention of disease-related complications. In the absence of family history, diagnosis in index cases is commonly considered at the time of the first or even the second major event only. Indeed, the rarity of the disease and its nonspecific physical signs do not favor early detection.[9] Consensual diagnostic criteria for EDS were proposed for the first time in 1986. VEDS was recognized as an independent disease and divided into 2 subtypes: the acrogeric and the ecchymotic forms. These criteria were further revised in 1997 (Villefranche nosology) to refine patient selection for genetic testing. Four major diagnostic criteria were identified: arterial/intestinal/uterine fragility or rupture, extensive bruising, thin and translucent skin, and characteristic facial appearance. The presence of 2 or more major criteria led to recommend molecular analysis. Several minor diagnostic criteria were also described to assist physicians in assessing clinically the likeliness of vEDS, but their diagnostic value other than indicative was not specified. Despite the recommended use of these criteria, their accuracy in patient diagnosis has never been evaluated.

To formally assess the diagnostic value of each clinical criterion, we designed a diagnostic accuracy study for the Villefranche criteria on all patients referred to our department, a dedicated referral center for vEDS, located in a tertiary hospital in Paris, France.

METHODS

For the purpose of transparency and openness, the data, analytic methods, and study materials are available at our center to other researchers for purposes of reproducing the results or replicating the procedures. A French ethics committee for noninterventional research approved this study (Institutional Review Board registration No. 00001072) in September 2016. An independent ethical research committee approved this study (Comité de Protection des Personnes, Ile de France II, IRB registration No. 00001072). Methods are now available in the Data Supplement.

RESULTS

Between January 2001 and March 2016, n=699 patients were referred to our center either for a suspicion of vEDS or for family screening (Figure 1). Primary motive of referral of index patients was arterial fragility and rupture. After exclusion of underage children and patients with incomplete medical records, n=519 patients remained eligible for analysis, predominantly index cases (Table 1). A pathogenic *COL3A1* variant was identified in n=165 (31.8%) patients, dividing into n=105 (27.3%) index patients and n=60 (44.4%) relatives. Patients were predominantly women (64.3% of probands and 61.5% of relatives). Index patients were younger than relatives at referral (mean age 38.6±13.5 versus 41.9±13.7 years, respectively; *P*=0.04). Considering the Villefranche classification, the mean number of major criteria was lower for relatives than for probands (*P*<0.01), as well as the mean number of minor criteria after exclusion of positive family history (*P*<0.001). Arterial/intestinal/uterine fragility or rupture was the most frequent diagnostic criterion for the index group and their primary referral motive (78.1%), without any sex difference (*P*=0.157). Extensive bruising was more commonly present in women (*P*=0.038), as well as gingival recession/fragility (*P*=0.025) and hyperlaxity of small joints (*P*<0.001). Conversely, pneumothorax and hemopneumothorax were more commonly present in men (10.0% versus 3.6%; *P*=0.005).

The patient's characteristics according to the absence or the presence of a *COL3A1* mutation are reported in Table 2. N=104 (63.0%) patients presented with glycine substitutions (group 1), n=42 (25.5%) had splice-site variants (group 2), n=15 (9.1%) patients had variants leading to haploinsufficiency (group 3), and n=4 belonged to group 4. Major diagnostic criteria such as thin, translucent skin, intestinal rupture/fragility, extensive bruising, and characteristic facial appearance were significantly over-represented in patients with positive

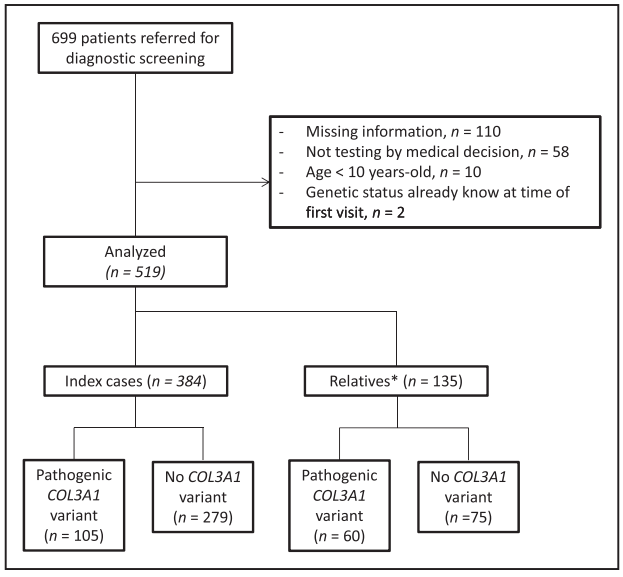

Figure 1. Study population.
*Out of n=135 relatives, n=21 were parents (9 with pathogenic *COL3A1* variant) and the remaining n=114 were sibs or cousins.

Table 1. Genetic Findings and Clinical Features as a Function of Sex and Index/relative Status

	Index			Relatives				
	Total	Men	Women	Total	Men	Women	*P* Value*	*P* Value†
N	384	137 (35.7%)	247 (64.3%)	135	52 (38.5%)	83 (61.5%)		
Age at referral	38.55	39.94	38.34	41.86	42.32	41.57	0.580‡	0.040‡
Variant								
No	279 (72.7%)	94 (68.6%)	185 (74.9%)	75 (55.6%)	27 (51.9%)	48 (57.8%)		
Yes	105 (27.3%)	43 (31.4%)	62 (25.1%)	60 (44.4%)	25 (48.1%)	35 (42.2%)	0.146§	<0.001§
Villefranche criteria								
Major diagnostic criteria (mean number)	1.86	1.94	1.82	0.90	0.73	1.01	0.930‡	<0.001‡
Thin, translucent skin	167 (43.5%)	67 (49%)	100 (40%)	40 (30%)	14 (27%)	26 (31%)	0.340§	0.006§
Arterial/intestinal/uterine fragility or rupture	300 (78.1%)	118 (86%)	182 (73.7%)	24 (17.8%)	8 (15.4%)	16 (19%)	0.157§	<0.001§
Arterial	271 (70.8%)	106 (77.4%)	165 (66.8%)	19 (14.8%)	7 (13.5%)	12 (14.5%)	0.205§	<0.001§
Intestinal	37 (9.6%)	16 (11.7%)	21 (8.5%)	5 (3.7%)	2 (3.8%)	3 (3.6%)	0.182§	0.046§
Uterine	11	0	11 (4.5%)	3	0	3 (3.6%)		0.989§
Extensive bruising	135 (35%)	41 (30%)	94 (38%)	30 (22%)	8 (15%)	22 (27%)	0.038§	0.007§
Characteristic facial appearance	114 (30%)	40 (29%)	74 (30%)	28 (21%)	8 (15%)	20 (24%)	0.511§	0.058§
Minor diagnostic criteria (mean number)	1.27	1.13	1.34	1.53	1.44	1.59	0.049‡	0.002‡
Acrogeria	78 (20%)	29 (21%)	49 (20%)	13 (10%)	3 (3%)	10 (12%)	0.878§	0.007§
Hypermobility of small joints	163 (42%)	42 (31%)	121 (49%)	25 (19%)	8 (15%)	17 (20%)	<0.001§	<0.001§
Tendon and muscle rupture	11 (3%)	5 (4%)	6 (2%)	1 (1%)	0	1 (1%)	0.765‖	0.200‖
Clubfoot	15 (4%)	9 (7%)	6 (2%)	5 (4%)	2 (4%)	3 (4%)	0.127§	1.000‖
Early-onset varicose veins	41 (11%)	11 (8%)	30 (12%)	14 (10%)	5 (10%)	9 (11%)	0.295§	0.949§
Arteriovenous, carotid-cavernous sinus fistula	8 (2.3%)	2 (2%)	6 (2.4%)	1 (1%)	0	1 (1%)	0.497‖	0.457‖
Pneumothorax/pneumohemothorax	27 (7%)	16 (12%)	11 (4%)	4 (3%)	3 (3%)	1 (1%)	0.005§	0.094‖
Gingival recession	46 (12%)	10 (7%)	36 (15%)	9 (7%)	2 (4%)	7 (8%)	0.025§	0.118§
Positive family history, sudden death in (a) close relative(s)	96 (25%)	30 (22%)	66 (27%)	135 (100%)	52 (100%)	83 (100%)	0.766§	<0.001§

*Men vs women.
†Index vs relatives.
Statistical analysis:
‡Student.
§χ^2 test and Yates correction.
‖Fisher exact test.

COL3A1 testing (*P*<0.001). Among minor diagnostic criteria, only hypermobility of small joints and gingival recession were not significantly associated with positive *COL3A1* testing (*P*=0.464 and *P*=0.125, respectively).

The Villefranche criteria were met for n=248 patients (209 index patients and 39 relatives; Appendix 4). For index cases, arterial/intestinal/uterine fragility or rupture was the most common major criterion (82.3%), whereas it was present in 46.1% of relatives only.

A pathogenic *COL3A1* variant was identified in n=131 of the 248 (52.8%) patients fulfilling the Villefranche criteria and in n=34 of the 71 (12.5%) patients not meeting the Villefranche criteria (Appendices 5, 6, and 7).

Patients not meeting the Villefranche criteria but with positive *COL3A1* testing were predominantly relatives (76.5%), and a majority of them had glycine-substitution variants (73.5%).

Another connective tissue disorder was identified in n=6 (2.5%) index patients not meeting the Villefranche criteria and without *COL3A1* variant (n=237 patients): kyphoscoliotic EDS (*PLOD1*, n=1), Loeys-Dietz syndrome type 1 (*TGFBR1*, n=1), Loeys-Dietz syndrome type 3 (*SMAD3*, n=2), and EDS hypermobile type (*TNXB*, n=2). Loeys-Dietz syndrome was suspected in 2 other probands, and 1 was clinically diagnosed with periodontal EDS, but died before genetic testing.[10] Molecular analyses were inconclusive for the remaining index cases: 109 presented an arterial event, 3 had intestinal fragility and 4 had uterine fragility. For the other patients, the original motive of referral was a suspicion of connective tissue disorder because of a combination of minor

Table 2. Baseline Characteristics of the Population According to the Presence or not of a Pathogenic COL3A1 Variant

	Genetic Variant		
	No	Yes	*P* Value
N	354	165	
Index/relative	279/75	105/60	
Men/women	121/233	68/97	
Mean age at referral	41.5	35.0	
Major diagnostic criteria			
Thin, translucent skin	97 (27.4%)	110 (66.7%)	<0.001*
Arterial/intestinal/uterine fragility or rupture	216 (61%)	109 (66.1%)	0.313*
Arterial	204 (57.6%)	88 (53.3%)	0.410*
Intestinal	9 (2.5%)	33 (20.0%)	<0.001*
Uterine (women only)	8 (2.3%)	6 (3.6%)	0.367†
Extensive bruising	71 (20.1%)	94 (57.0%)	<0.001*
Characteristic facial appearance	40 (11.3%)	102 (61.8%)	<0.001*
Minor diagnostic criteria			
Acrogeria	23 (6.5%)	68 (41.2%)	<0.001*
Hypermobility of small joints	124 (35%)	64 (38.8%)	0.464*
Tendon and muscle rupture	4 (1.1%)	8 (4.8%)	0.022†
Clubfoot	3 (0.8%)	17 (10.3%)	<0.001†
Early-onset varicose veins	21 (5.9%)	34 (20.6%)	<0.001*
Arteriovenous, carotid-cavernous sinus fistula	2 (0.6%)	7 (4.2%)	0.006†
Pneumothorax/ pneumohemothorax	13 (3.7%)	18 (10.9%)	0.002*
Gingival recession	32 (9%)	23 (13.9%)	0.125*
Positive family history, sudden death in (a) close relative(s)	128 (36.2%)	103 (62.4%)	<0.001*

Statistical analysis:
*χ^2 test and Yates correction.
†Fisher exact test.

signs or in the presence of extensive bruising. For the remaining relatives (n=70), systematic family screening revealed no pathogenic *COL3A1* variant.

In the whole study population, the Villefranche criteria had a sensitivity of 79% (95% CI, 0.72–0.85) and a specificity of 67% (95% CI, 0.62–0.72; Table 3). In this population of selected patients, ie, with a high prevalence of vEDS, overall negative predictive value (NPV) was 87% (95% CI, 0.83–0.91). However, this value was significantly influenced by the proband group, which had better sensitivity and NPV (92% and 95%, respectively). Indeed, diagnostic odds-ratio (DOR) values were 18.1 (95% CI, 8.46–38.7) for index cases versus 7.8 (95% CI, 5.04–12.09) in the whole cohort. Notably, sensitivity and NPV were lower for young patients with the worst DOR for probands aged 25 years or less (5.1; 95% CI, 1.41–18.7). Conversely, the highest specificity and positive predictive value (PPV) were observed in relatives (specificity, 0.93; CI 95%, 0.85–0.98; PPV 0.87; CI 95%, 0.73–0.96).

When applying the 2017 vEDS diagnostic criteria to the whole cohort, diagnostic accuracy was lower than that of the Villefranche classification (Tables 3 and 4). Notably, sensitivity and NPV were less effective for probands (68% and 84%, respectively), with a lower DOR (4.04; 95% CI, 2.51–6.52). As expected with more stringent diagnostic criteria, specificity and PPV were higher than those of the Villefranche criteria. As for the latter, young patients seemed least fit for accurate vEDS detection (DOR, 2.36; 95%CI, 0.80–6.90). Criteria with the highest DOR were intestinal rupture, characteristic facial appearance, acrogeria, clubfoot, and arteriovenous or carotid-cavernous sinus fistula (not shown). Establishing a cutoff number of major and minor criteria proved far less effective in any aspects (sensitivity, specificity, PPV, and NPV), than the Villefranche classification (not shown).

Diagnostic accuracy of each individual criterion for index cases is reported in Table 5. Major diagnostic criteria of the Villefranche classification present a high sensitivity ranging from 67% for extensive bruising to 84% for organ fragility unlike minor criteria, for which sensitivity is lower. Adversely, specificity is high for minor criteria ranging from 81% for existence of a positive family history of vEDS, to 99% for tendon or muscle rupture, clubfoot or arteriovenous sinus fistula (with the exception of hypermobility of small joints: 49%; 95% CI, 0.53–0.65). DOR's were high for components of the acrogeric phenotype: characteristic facial appearance (14.3; 95% CI, 8.34–24.4) and acrogeria (13.4; 95% CI, 7.47–23.9). Unsurprisingly, clubfoot had the highest DOR (19.6; 95% CI, 4.33–88.4) and bowel perforation in association with arterial fragility was highly diagnostic (10.9; 95% CI, 4.94–24.1). For relatives, overall sensitivity of diagnostic criteria is lower, whereas specificity is higher (Appendix 8). The 2017 International Classification proposed novel or modified criteria, such as congenital hip dislocation or a combination of gingival recession and fragility. In our cohort, congenital hip dislocation was present in 8 patients only, of which 3 had a pathogenic *COL3A1* variant (sensitivity 1.8% and specificity 98.6%). Gingival recession and fragility was present in 25 patients, of which 9 had confirmed vEDS (sensitivity 5.5% and specificity 95.5%; not shown).

DISCUSSION

Main Results

In a specialized setting, the Villefranche diagnostic criteria prove to be reliable for indicating genetic testing in patients with a suspicion of vEDS, especially in symptomatic adult probands. On the contrary, the Villefranche criteria appear to be least accurate in presymptomatic index patients (children, teenagers, and young adults). Significant differences in performance of the

Table 3. Diagnostic Accuracy of the Villefranche Classification

	Se (CI 95%)	Sp (CI 95%)	PPV (CI 95%)	NPV (CI 95%)	DOR (CI 95%)
Whole population*	0.79 (0.72–0.85)	0.67 (0.62–0.72)	0.53 (0.46–0.59)	0.87 (0.83–0.91)	7.8 (5.04–12.09)
Index*	0.92 (0.85–0.97)	0.60 (0.54–0.66)	0.46 (0.39–0.53)	0.95 (0.91–0.98)	18.1 (8.46–38.7)
Index women†	0.93 (0.84–0.98)	0.60 (0.53–0.67)	0.44 (0.35–0.53)	0.96 (0.91–0.99)	21.8 (7.57–62.5)
Index men†	0.91 (0.78–0.97)	0.60 (0.49–0.70)	0.51 (0.39–0.62)	0.93 (0.84–0.98)	14.4 (4.74–43.5)
Index ≥40 y-old†	0.92 (0.75–0.99)	0.58 (0.49–0.66)	0.28 (0.19–0.39)	0.98 (0.92–1.00)	16.3 (3.72–71.7)
Index ≤25 y-old†	0.85 (0.6–0.96)	0.48 (0.29–0.67)	0.59 (0.42–0.75)	0.79 (0.52–0.94)	5.1 (1.41–18.7)
Relatives*	0.57 (0.43–0.69)	0.93 (0.85–0.98)	0.87 (0.73–0.96)	0.73 (0.63–0.81)	18.3 (6.46–51.9)
Relatives women*	0.66 (0.48–0.81)	0.90 (0.77–0.96)	0.82 (0.63–0.94)	0.78 (0.65–0.88)	16.5 (5.17–52.6)
Relatives men†	0.44 (0.24–0.65)	1.00 (0.87–1.00)	1.00 (0.71–1.00)	0.66 (0.49–0.80)	43.6 (2.39–795)

DOR indicates diagnostic odds-ratio; Se, Sensitivity; Sp, Specificity; PPV, Predictive positive value; and NPV, Negative predictive value.
Statistical analysis:
*χ^2 test and Yates correction.
†Fisher exact test.

major and minor diagnostic criteria according to index/relative status were found. The new 2017 classification maintain partly the same clinical criteria with addition of some others, specific but of notably low sensitivity. It is, therefore, unsurprising that its overall diagnostic performance is lower than that of the Villefranche criteria. Despite highly specific diagnostic criteria, diagnostic accuracy of presymptomatic index patients also remains unsatisfactory.

Interpretation of Results

There is a substantial difference between someone being referred for a suspicion of connective tissue disorder after a meaningful medical/surgical event or in the context of outstanding physical signs, and patients being referred for genetic screening because of the identification of a genetic condition in a family member. In our study, sensitivity and NPV of the Villefranche criteria are lower for relatives than in other groups. However, in this specific situation, genetic testing is mandatory and diagnostic criteria are of minimal importance.

For index cases, sensitivity and NPV are relatively high making these criteria a good diagnostic tool. However, differences in accuracy exist for sex and age. The difference of performance between men and women might be explained by under-referral of men because of premature death or a less marked acrogeric phenotype.[11] Behavioral differences between men and women might also be hypothesized. Differences according to age can simply be explained by the criteria themselves, since they were designed to detect primarily symptomatic patients. Indeed, the Villefranche criteria take into consideration a history of arterial or intestinal or uterine events. Probability of such accidents inevitably increases with age. As a consequence, sensitivity is highest in probands after 40 years (Figure 2). For physical signs of vEDS or congenital defects, there is no argument for a higher prevalence in older patients than in the younger ones. Because of their high specificity, most minor criteria are of diagnostic interest when present, particularly for probands with a history of clubfoot.

Study Limitations

Given that our patients are most commonly being referred in a context of spontaneous arterial accidents, we were not able to determine precisely the diagnos-

Table 4. Diagnostic Accuracy of the 2017 International EDS Classification

	Se (CI 95%)	Sp (CI 95%)	PPV (CI 95%)	NPV (CI 95%)	DOR (CI 95%)
Whole population*	0.79 (0.72–0.85)	0.52 (0.47–0.57)	0.43 (0.39–0.49)	0.84 (0.79–0.89)	4.17 (2.71–6.42)
Index*	0.68 (0.58–0.76)	0.66 (0.60–0.71)	0.43 (0.35–0.51)	0.84 (0.79–0.89)	4.04 (2.51–6.52)
Index women*	0.66 (0.53–0.78)	0.65 (0.57–0.72)	0.39 (0.29–0.49)	0.85 (0.78–0.90)	3.60 (1.97–6.61)
Index men*	0.70 (0.54–0.83)	0.68 (0.58–0.77)	0.50 (0.37–0.63)	0.83 (0.73–0.91)	4.92 (2.25–10.76)
Index ≥40 y-old†	0.19 (0.07–0.39)	0.94 (0.88–0.97)	0.36 (0.13–0.65)	0.86 (0.80–0.91)	3.57 (1.09–11.59)
Index ≤25 y-old*	0.58 (0.37–0.77)	0.65 (0.46–0.82)	0.60 (0.39–0.79)	0.63 (0.44–0.80)	2.36 (0.80–6.90)

Diagnostic accuracy according to available criteria, including arterial rupture/fragility, digestive rupture/fragility, uterine rupture/fragility, excluding congenital hip dislocation, and keratoconus. DOR indicates diagnostic odds-ratio; Se, Sensitivity; Sp, Specificity; PPV, Predictive positive value; and NPV, Negative predictive value.
Statistical analysis:
*χ^2 test and Yates correction.
†Fisher exact test.

Table 5. Diagnostic Accuracy of Each Criterion of the Villefranche Classification for Probands

	Se (CI 95%)	Sp (CI 95%)	PPV (CI 95%)	NPV (CI 95%)	DOR (CI 95%)
Major criteria					
Thin, translucent skin*	0.73 (0.64–0.81)	0.68 (0.62–0.73)	0.46 (0.38–0.54)	0.87 (0.82–0.91)	5.78 (3.50–9.52)
Arterial/intestinal/uterine fragility or rupture*	0.84 (0.75–0.90)	0.24 (0.19–0.29)	0.29 (0.24–0.35)	0.80 (0.70–0.88)	1.64 (0.91–2.94)
Arterial*	0.67 (0.57–0.76)	0.28 (0.23–0.34)	0.26 (0.21–0.31)	0.69 (0.60–0.77)	0.78 (0.48–1.26)
Intestinal*	0.27 (0.18–0.36)	0.97 (0.94–0.98)	0.76 (0.59–0.88)	0.78 (0.73–0.82)	10.9 (4.94–24.1)
Uterine (women only, n=247)†	0.06 (0.02–0.16)	0.96 (0.92–0.98)	0.36 (0.11–0.69)	0.75 (0.69–0.81)	1.75 (0.49–6.21)
Extensive bruising*	0.67 (0.57–0.76)	0.77 (0.71–0.81)	0.52 (0.43–0.60)	0.86 (0.81–0.90)	6.58 (4.03–10.8)
Characteristic facial appearance*	0.70 (0.61–0.79)	0.86 (0.81–0.90)	0.65 (0.55–0.74)	0.88 (0.84–0.92)	14.3 (8.34–24.4)
Minor criteria					
Acrogeria*	0.53 (0.43–0.63)	0.92 (0.88–0.95)	0.72 (0.60–0.81)	0.84 (0.79–0.88)	13.4 (7.47–23.9)
Hypermobility of small joints*	0.46 (0.36–0.56)	0.59 (0.53–0.65)	0.29 (0.23–0.37)	0.74 (0.68–0.80)	1.20 (0.76–1.89)
Tendon and muscle rupture†	0.07 (0.03–0.13)	0.99 (0.96–1.00)	0.64 (0.31–0.89)	0.74 (0.69–0.78)	4.91 (1.41–17.1)
Clubfoot†	0.12 (0.07–0.20)	0.99 (0.97–1.00)	0.87 (0.59–0.98)	0.75 (0.70–0.79)	19.6 (4.33–88.4)
Early-onset varicose veins*	0.22 (0.14–0.31)	0.93 (0.90–0.96)	0.56 (0.40–0.71)	0.76 (0.71–0.80)	4.07 (2.09–7.91)
Arteriovenous, carotid-cavernous sinus fistula†	0.06 (0.02–0.12)	0.99 (0.97–1.00)	0.75 (0.35–0.97)	0.74 (0.69–0.78)	8.39 (1.67–42.3)
Pneumothorax/pneumohemothorax*	0.14 (0.08–0.22)	0.96 (0.93–0.98)	0.56 (0.35–0.74)	0.75 (0.70–0.79)	3.71 (1.67–8.22)
Gingival recession*	0.15 (0.09–0.24)	0.89 (0.85–0.93)	0.35 (0.21–0.50)	0.74 (0.69–0.78)	1.49 (0.78–2.87)
Positive family history, sudden death in (a) close relative(s)*	0.41 (0.31–0.51)	0.81 (0.76–0.85)	0.45 (0.35–0.55)	0.78 (0.73–0.83)	2.96 (1.81–4.83)

DOR indicates diagnostic odds-ratio; Se, Sensitivity; Sp, Specificity; PPV, Predictive positive value; and NPV, Negative predictive value.
Statistical analysis:
*χ^2 test and Yates correction.
†Fisher exact test.

tic accuracy of arterial rupture/fragility (Table 2, similar rates of arterial fragility/rupture in *COL3A1* positive and negative patients). However, recurrent spontaneous arterial accidents in probands were not significantly associated with the presence of *COL3A1* mutations in the absence of other major and minor criteria. Other diagnostic values of major criteria as digestive and uterine rupture/fragility may also be underestimated because of a referral bias or simply because of the rarity of the considered event (uterine rupture).

To assess the presence or the absence of each criterion, information was collected from the visit report and the patient's medical file. A criterion was considered absent when not mentioned. There were no standardized reports, so each sign was not systematically reported. However, validity of collected information was verified and compared per physician (Appendix 9). Major criteria were rarely omitted but despite excellent overall comparability, minor criteria might have been underestimated. Subjective diagnostic criteria not

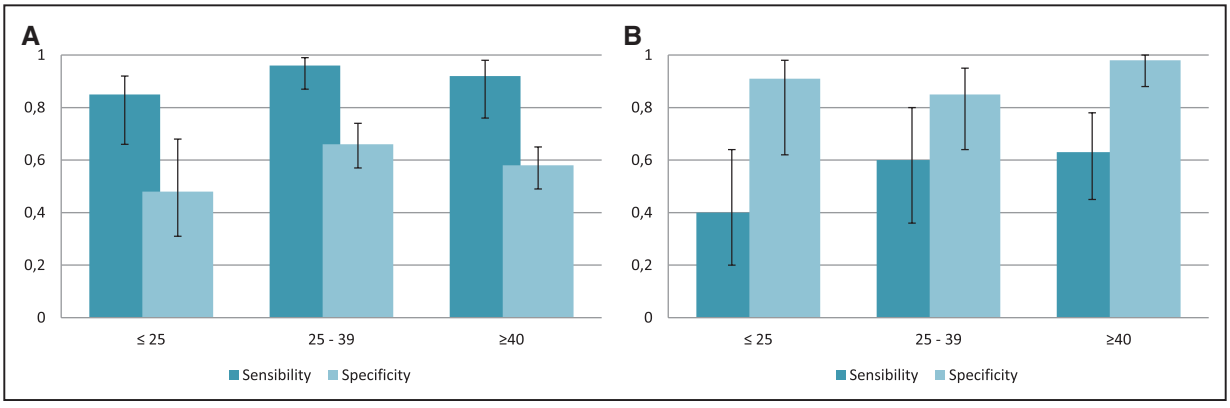

Figure 2. Accuracy of the Villefranche criteria according to the age of first consultation.
A, For index patients, n=55, 159, and 170 for patients ≤25y, 25 to 39y, and ≥40y, respectively. **B**, For relatives, n=26, 35, and 74 for patients ≤25y, 25 to 39y, and ≥40y, respectively.

having a formal definition nor specific staging (skin translucency or acrogeric traits for example) were likely variably appreciated, depending mostly on the attending physician's personal experience. There is no indication however that this limitation might significantly alter the Villefranche criteria's diagnostic performance. Interestingly, concise medical reports were more frequent when the physician had no doubt about the absence or the presence of the pathology.

Because of the type of data collection that was performed and due to the decision to perform a molecular analysis in the presence of evocative criteria, NPV and PPV are probably more reliable than sensitivity and specificity even if they need to be interpreted considering the particular setting of this study.

Finally, as these criteria have been used and evaluated in a highly specialized setting, our results may be considered valid for use in some specialized diagnostic settings only.

Regarding the 2017 criteria, our findings intended to be a comprehensive approach only based on the criteria already present in the Villefranche classification.

Literature Review

Although there are not many formal reports on the prevalence of minor criteria, reported characteristics of this cohort do not differ from previous reported patient cohorts. In their review, De Paepe and Malfait found that 60% of probands were referred for molecular analysis after 1 or more major complication and that 40% were referred because evocative clinical features.[1] In our study, diagnostic outpatient referral after a major complication seems to be more predominant for index patients (78%). Pepin et al[12] presented a clinical review with a large number of patients, both probands and relatives. Mean age at ascertainment was almost 10 years lower than in our study for probands (24.9 versus 33.5 years for *COL3A1* positive index cases). This difference is likely explained by us being a dedicated clinical referral and care center for patients generating a specific type of diagnostic referral. The level of clinical expertise of physicians referring samples or patients in the Pepin et al[12] cohort is not known. Nonetheless, consistent presentations were observed in both centers: arterial rupture/fragility was the first complication in 46% of probands and gastrointestinal rupture/fragility for 19%, versus 53% and 20% respectively in our center. Similarly, clubfoot had been identified in 9.8% of subjects, versus 12% in our cohort. Gingival recession was identified in 15% in the presence and in 10.7% in the absence of a *COL3A1* pathogenic mutation. Ferré et al[13] estimated its prevalence in a dedicated odontal clinic at 41.2% of vEDS patients, but also in 67.3% of healthy controls, likely explaining its low diagnostic weight. Gingival fragility, more easily and reliably identifiable by nondental health professionals might thus be a more discriminant diagnostic criterion than recession. Early onset varicose veins were identified in 20.6% in the presence and in 5.9% in the absence of a pathogenic *COL3A1* mutation. This result is concordant with the fact that superficial venous insufficiency is reportedly more prevalent in vEDS patients (37%) than in the general population (17–23%).[14]

Towards a More Selective Application of Diagnostic Criteria

In our cohort—where patients were tested with 2 or more major diagnostic criteria and also in the context of arterial fragility alone—a pathogenic *COL3A1* variant was identified in 27.3% of probands. If the Villefranche criteria had been strictly applied (indication of molecular analysis only for patients with 2 major criteria or more) this value would have gone up to 46.4%, resulting in a substantial cost reduction, but in return, 2.1% of our index patients would not have been diagnosed. On the one hand, this may seem an acceptable number, especially since patients with organ fragility are likely to have a dedicated follow-up. On the contrary, the absence of diagnosis of this life-threatening inherited disease even in few patients might seem unacceptable not only for the patient requiring a specific care and follow-up but also for his/her at-risk relatives for whom an early-onset diagnosis might allow prevention of complications.

Until recently, the revised Villefranche nosology was intended to accurately detect and select patients for genetic testing, partly because of the technical difficulty of the molecular/biochemical characterization of *COL3A1* mutations at the time it was designed. Technical progress in genetics now allows easier, faster, and more reliable screening of the *COL3A1* gene and others.[15] Hence, the 2017 revised diagnostic criteria provide no cutoff number of criteria for genetic testing, and evocative clinical criteria have been incremented in the likely intention of broadening clinical screening of possibly affected patients. However, the narrowing of major criteria, as well as the addition of minor criteria of low prevalence (congenital hip dislocation and keratoconus) negatively affects sensitivity, and ultimately the overall diagnostic accuracy of the revised criteria. Thus to our view, the Villefranche nosology should remain the primary diagnostic criteria for vEDS in specialized practice.

Genetic testing is a mandatory step for formal diagnosis of vEDS. Medical history and physical signs should help accurate selection of patients at risk and to avoid false negatives. In this respect, clinical criteria should have both a high sensitivity and a high NPV. In clinical practice, diagnostic criteria primarily apply for probands. Indeed, for the genetic screening of relatives of

an affected individual, diagnostic criteria are not needed to determine whether the subject should be tested or not. For symptomatic probands (patients with1 major criterion: organ fragility), the most effective diagnostic criteria (2nd major and minor criteria) were dermatologic findings (skin translucency and bruising), acrogeric traits (characteristic facial appearance and acrogeria), clubfoot, gingival fragility/recession, and early onset varicose veins.

The Villefranche diagnostic criteria primarily address the detection and clinical diagnosis of symptomatic vEDS patients, typically adults in their early thirties. The adult-onset of organ complications in vEDS explain why the Villefranche criteria perform poorly in children, teenagers, and adults aged <24 years. In this group of patients, diagnostic criteria as easy bruising, acrogeric traits, and clubfoot seem to be of particular interest, particularly in a context of spontaneous pneumothorax.

CONCLUSIONS

This study is to the best of our knowledge, the first formal evaluation of the diagnostic accuracy of clinical diagnostic criteria for vEDS. In specialized practice, the Villefranche criteria seem to be an effective diagnostic tool, particularly for symptomatic adult probands. The presence of 2 or more major criteria are an accurate cutoff for indicating genetic testing, but with limited specificity. The revised diagnostic criteria of 2017 were found to be less accurate in any tested aspects. Their interest in clinical practice, even as a screening tool, remains to be determined. Finally, the detection of presymptomatic children, teenagers, and young adults without a family history of vEDS remains unadressed by both diagnostic classifications.

ARTICLE INFORMATION

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Disclosures

None.

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