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La Revue de Médecine interne – Article Original

Characteristics of Systemic Sclerosis patients with positive anti-Th/To antibodies: about 6 patients and literature review

Caractéristiques des patients souffrant de Sclérodémie Systémique avec positivité des anticorps anti-Th/To : au sujet de 6 patients et revue de la littérature

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2

3 **Résumé**

4

5 Parmi les anticorps décrits associés à la Sclérodémie Systémique (ScS), les anticorps anti-
6 Th/To (anti-Th/To) sont rares et ont été peu étudiés. Ainsi, nous n'avons que peu de
7 connaissance sur le profil des patients positifs pour les anti-Th/To. A partir d'une Biobanque
8 locale (Marseille, France), nous avons sélectionné de façon rétrospective 6 patients positifs
9 pour les anti-Th/To, en test Immunodot. Tous souffraient de ScS, et partageaient des
10 caractéristiques cliniques et biologiques communes comme la forme cutanée limitée de la
11 ScS, une baisse de la diffusion alvéolocapillaire pulmonaire et un aspect moucheté nucléolaire
12 en immunofluorescence indirecte au test de dépistage des anticorps anti-nucléaires sur cellules
13 Hep-2.

14 Afin de caractériser davantage les patients positifs pour les anti-Th/To, nous avons réalisé une
15 revue de la littérature sur le sujet. A partir de l'étude de 402 patients positifs pour les anti-
16 Th/To, nous confirmons que ces anticorps sont principalement associés à la forme cutanée
17 limitée de la maladie (88% de l'ensemble des patients), et à une atteinte pulmonaire de la ScS
18 (50% de l'ensemble des patients). Cette analyse de la littérature illustre la rareté des anti-
19 Th/To avec une fréquence moyenne estimée de 3,4% chez tous les patients souffrant de ScS
20 dans le monde entier, leur caractère habituellement exclusif vis-à-vis des autres autoanticorps
21 spécifiques de la Sclérodémie, ainsi que leur grande spécificité (environ 98%) pour le
22 diagnostic de la ScS.

23

24

25 Mots clé : Sclérodémie systémique; anti-Th/To autoanticorps; forme cutanée limitée; atteinte
26 interstitielle pulmonaire

27

28

29

30 **Abstract:**

31

32 Among the antibodies described in Systemic Sclerosis (SSc), anti-Th/To antibodies (anti-
33 Th/To) are rare and have been poorly studied. Thus, little is known about the profile of anti-
34 Th/To positive patients. From our local Biobank (Marseille, France), we retrospectively
35 selected data for 6 patients positive for anti-Th/To with an Immunodot assay. All of them
36 suffered from SSc, sharing clinical and biological common features such as a limited
37 cutaneous form of SSc, a decreased lung diffusing capacity and a speckled nuclear nucleolar
38 immunofluorescence pattern of antinuclear antibodies screening on HEp-2 cells.

39 In order to further characterize patients positive for anti-Th/To, we performed a thorough
40 literature review. From 402 studied patients positive for anti-Th/To, we confirmed that these
41 antibodies are associated with the limited cutaneous form of the disease (88% of the patients),
42 and with an SSc related-pulmonary involvement (50%). The review analysis pointed out the
43 rarity of the anti-Th/To with an estimated mean frequency of 3.4% of all SSc patients
44 worldwide, their usual exclusivity with respect to the specific antibodies of scleroderma, and
45 their high specificity (around 98%) for the diagnosis of SSc.

46

47 Keywords: Systemic Sclerosis; anti-Th/To antibodies; limited cutaneous form; interstitial lung
48 disease

49

50

51 **Introduction**

52

53 Systemic sclerosis (SSc, scleroderma) is a rare connective-tissue disease characterized by skin
54 and internal organ fibrosis, vascular alteration and a specific auto-immune profile [1,2]. For
55 patients suspected to suffer from scleroderma, based on clinical and morphological features,
56 characterization of their immunological profile offers a crucial load in the final diagnosis. To
57 date, three autoantibodies have been considered as specific of SSc: the anti-topoisomerase I
58 (anti-topo 1), anti-centromere (ACA) and anti-RNA Polymerase III (anti-RNAPolIII)
59 antibodies. Clinical characteristics and prognosis associated with anti-topo 1, ACA and anti-
60 RNAPolIII antibodies are well characterized [1]. Indeed, anti-RNAPolIII antibodies are
61 mainly associated with a diffuse cutaneous subtype, a higher risk of scleroderma renal crisis
62 and a risk of cancer in close temporal relationship to SSc onset [3-6]. Anti-topo1 antibodies
63 are usually associated with a diffuse cutaneous form, pulmonary fibrosis, severe peripheral
64 vascular disease and cardiac involvement, all resulting in a high mortality rate [7-10]. On the
65 contrary, anti-centromere (ACA) are associated with the limited cutaneous type, and are
66 protective for Interstitial Lung Disease (ILD), cardiomyopathy, and death [3,11,12]. Other
67 antibodies associated with SSc such as anti-Th/To are rarely observed and their impact on
68 clinical characteristics and prognosis have been poorly described [1,2,3].

69 Th/To antigens are located in the nucleolus of the cell nucleus. Anti-Th/To antibodies
70 recognize two endoribonucleases: the ribonucleoprotein 7-2/MRP-RNP (Th) and the
71 ribonuclease 8-2/RNP (To); the antigenic complex is 40kD. They display a nucleolar staining
72 by indirect immunofluorescence on HEp-2 cells.

73 In this article, we report the characteristics of 6 French patients positive for anti-Th/To, and
74 we propose a review of the clinical characteristics associated with this type of antibody.

75

76 **Patients and methods:**

77 Sera were collected from a Biobank (DC 2012-1704) with respect of ethical directives in the
78 laboratory of immunology of APHM in Marseilles (South of France). Antinuclear antibodies
79 (ANAs) were detected by indirect immunofluorescence on HEp-2 cells (Bio-Rad
80 Laboratories, Hercules, CA) at a screening dilution of 1:160.

81 Over a one-year period (January 2017 to December 2018), we selected 64 samples positive
82 for ANAs associated with a clinical suspicion of SSc and without positivity of specific SSc
83 autoantibodies (anti-topo 1, ACA and anti-RNAPolIII). These sera were tested for anti-Th/To

84 IgG antibodies using BlueDiver® Combi Immunodots (Alphadia SA/SN, Wavre, Belgium)
85 commercialized by Eurobio-Ingen®, Les Ulis, France.

86 We retrospectively collected the following clinical data on patient's medical records: i/
87 clinical characteristics of patients positive for anti-Th/To comprised demographic
88 characteristics (age, sex), ACR/EULAR 2013 criteria fulfillment, associated diagnosis in case
89 of overlap syndromes, Raynaud's phenomenon, sclerodactyly, calcinosis, digital pitting scar,
90 and telangiectasias. Upper digestive signs included esophageal reflux, dysphagia, or
91 dyspepsia; lower digestive signs included malabsorption, diarrheas, or anal incontinence.
92 Pulmonary function tests (Total Lung Capacity, DLCO) and echocardiography (Left Ejection
93 Ventricular Fraction, pericardial effusion, Pulmonary Arterial Hypertension) were recorded.
94 Interstitial Lung disease (ILD) on chest CT scan was defined by with qualitative criteria
95 consisting in the presence of honey combing, ground glass opacities, reticular abnormalities,
96 traction bronchiectasis and septa thickening. Muscle involvement was defined by muscular
97 pain or CPK elevation. ii/ immunological parameters were also recorded: ANAs, anti-dsDNA.
98 ACA/anti-RNAPol III/anti-topo I, anti-U1-RNP, anti-SSA/SSB, anti-Jo1/PM-Scl, anti-CCP,
99 Rheumatoid factor level, anti-mitochondrial, Lupus Anticoagulant and antiphospholipid
100 antibodies, cryoglobulinemia.

101 Secondly, we questioned the research banks Pubmed and Google scholar, with keywords
102 "scleroderma", "systemic scleroderma", "auto-antibodies profiles", "Th/To" in order to
103 collect a large population of patients anti-Th/To positive and gather their clinical and
104 phenotypic characteristics. We then extracted statistical information regarding these
105 antibodies.

106 For the statistical analysis, weighted arithmetic means were calculated from the literature
107 review to estimate the frequency of Th/To antibodies among SSc patients, and the frequency
108 of limited cutaneous form or pulmonary involvement among Th/To patients. Weighted
109 arithmetic means were obtained by weighting for each study the result of the frequency of
110 analyzed data by the number of included patients.

111

112 **Results:**

113 **1) Clinical and biological characteristics of 6 anti-Th/To antibody positive patients** 114 **from the BioBank**

115 Among the 64 sera referred to the laboratory for the routine detection of SSc-related
116 autoantibodies, 6 were found positive for anti-Th/To and negative for the SSc classical
117 autoantibodies (anti-topo 1, ACA and anti-RNAPolIII). The 6 patients were diagnosed with
118 SSc according to the 2013 ACR/EULAR criteria. **Table 1** shows their characteristics.

119
120 The six patients shared clinical and biological characteristics. The main clinical features
121 included: Raynaud's syndrome (6/6) and sclerodactyly (6/6), telangiectasia (6/6), gastro
122 esophageal reflux (5/6), digital pitting scar (2/6) and calcinosis cutis (1/6). All of these
123 patients had a cutaneous limited form. Moreover, all of the six sera displayed positive ANAs
124 with a speckled nuclear nucleolar immunofluorescence pattern (**Figure 1**), corresponding to
125 the nucleolar localization of the antigen Th/To.

126
127 Two patients had auto-immune associated diseases: Sjogren's syndrome (patient 1),
128 characterized by a sicca syndrome, typical salivary glands biopsy histological analysis and
129 anti-SSA (60 KD) antibodies, and primary biliary cirrhosis (patient 2) with anti-mitochondrial
130 antibody and a mixed cryoglobulinemia.

131 None of the patients had a restrictive pulmonary syndrome, but all had a decreased diffusing
132 lung capacity of carbon monoxide (DLCO), and 5 had an ILD on chest CT-scan. The
133 echocardiography noted a normal left ventricular ejection fraction (6/6), pulmonary arterial
134 hypertension (PAH) (1/6 confirmed with a right heart catheterization), and pericarditis (2/6).
135 One patient suffered from myalgia with normal CPK levels.

136 Three anti-Th/To positive patients also had anti-SSA (60 kD) antibodies. When performed
137 ($n=3$), the search for anti-phospholipid antibodies was negative.

138

139 ***2) Literature review on anti-Th/To antibodies***

140

141 Results of the literature research regarding the positivity of anti-Th/To antibodies in SSc
142 patients are summarized in **Tables 2-4**.

143

144 Firstly, in order to estimate the frequency of anti-Th/To positivity in SSc patients, we
145 collected the data from 25 articles including 8727 SSc patients (**Table 2**): a total of 300
146 patients were found positive for anti-Th/To antibodies. The estimated frequency of anti-Th/To
147 among SSc patients ranged between 0 to 13%, with a mean of 3.4% (300/8727, median = 3%,
148 first and third interquartile = 2 and 4%). The positivity of anti-Th/To did not seem to be

149 affected by the dosage method, since 3.3% of the patients were positive using a DOT and
150 3.4% using radioimmunoprecipitation.

151
152 Secondly, we also studied the frequency of anti-Th/To according to the continent on which
153 the studies were carried out (**Table 3**). Interestingly, these antibodies seem to be more
154 frequent in Asia and Oceania (4.7%) than in Europe (1.5%). It is difficult to determine the
155 frequency of anti-Th/To in South America from a single study.

156
157 Literature review on the cutaneous form among SSc patients with anti-Th/To antibodies,
158 based on 313 patients from 16 articles, highlighted the high frequency of the limited
159 cutaneous form, respecting 87.5% of the patients (median = 89%, from 55% to up to 100%
160 depending of the case series).

161
162 Finally, in order to estimate the frequency of pulmonary fibrosis among SSc patients with
163 anti-Th/To, we collected the data from 10 articles including 264 SSc patients with anti-Th/To
164 (**Table 4**). Pulmonary involvement was screened with different methods; chest radiography,
165 chest CT scan, or pulmonary function testing (PFT). As example, PFT could be considered
166 pathological either by an anomaly of the DCLO < 75%, or a total lung capacity (TLC) < 80%,
167 or sometimes not specified at all in the article. In this review, 47.3% of SSc patients with anti-
168 Th/To suffered from pulmonary fibrosis (median = 42%, first and third interquartile = 35 and
169 53%).

170

171 **Discussion**

172
173 We report here 6 patients diagnosed with an SSc for whom the only immunological marker
174 for the SSc disease was the positivity of anti-Th/To. The presence of anti-SSA (60kD)
175 antibody was found in 3/6 patients. One patient completed the international classification
176 criteria for Sjögren's syndrome [13] and was diagnosed with an overlap syndrome. Another
177 patient suffered from an associated auto-immune cholangitis. The association between SSc
178 and auto-immune cholangitis has been described as Reynold's syndrome [14].

179 In our series, all of the patients had a limited cutaneous form of the disease, and most of them
180 had interstitial lung disease and a decreased DLCO value. Consistently, the literature review

181 revealed an association between anti-Th/To and the limited cutaneous form of the disease and
182 the ILD, defining a specific clinical pattern or phenotype associated with this antibody.

183

184 ACA represent the main antibodies associated with the limited cutaneous form of SSc. Thus,
185 the comparison of clinical and prognostic characteristics of anti-Th/To and ACA patients
186 appears interesting. Mitri et al. [15] described the characteristics of 96 anti-Th/To positive
187 patients in the larger cohort reported in literature. They concluded that, in comparison to ACA
188 positive patients, anti-Th/To positive patients had a lower cutaneous extension of skin
189 sclerosis.

190 Our literature review also suggested less digital pitting scars, digital tip ulcers, and gangrene
191 in anti-Th/To positive SSc patients [16-21], provided that such clinical manifestations were
192 heterogeneously assessed based on scleroderma skin score (Rodnan Scleroderma Skin Score,
193 Cochin Hand score, Kapandji index..), Raynaud's phenomenon severity score, frequency of
194 ulcers or digital scars. Conversely, ACA are known to be related with a high risk of severe
195 digital vascular disease [3,11,12] .

196

197 Mitri et al. [15] also noticed that anti-Th/To antibodies were associated with a higher risk of
198 ILD (47%) compared to ACA (13%). This result was supported by Ceribelli et al. [19] in a
199 smaller cohort of patients. Our literature review estimated that half of anti-Th/To positive
200 patients have a pulmonary involvement. This percentage was higher than the one found in the
201 literature for ACA which was around 30-40% [3,21].

202 Interestingly, anti-Th/To have been retrospectively detected in 13/285 patients with a clinical
203 diagnosis of idiopathic pulmonary fibrosis [3,21]. In this study, among the 13 anti-Th/To
204 positive patients, 9 were retrospectively diagnosed as having SSc *sine scleroderma*, based on
205 immunologic characteristics, Raynaud's phenomenon and pulmonary involvement. This latter
206 article highlighted a clinical pattern associated with anti-Th/To, characterized by a high
207 frequency of pulmonary disease, mild skin sclerosis and peripheral vascular involvement.

208

209 The specificity of anti-Th/To for the diagnosis of SSc appears very high, estimated between
210 90 and 99% from articles assessing the anti-Th/To among cohorts of scleroderma and non-
211 scleroderma patients. Indeed, Kuwana et al [22] found that anti-Th/To had a 99% specificity
212 for SSc diagnosis among a cohort of 1048 patients with various auto-immune diseases.
213 Similar results were obtained in Villalta et al [23], Van Praet et al [24] and Mahler et al [20],
214 with a specificity of respectively 99, 98 and 99.5%. In Mitri et al [15], the specificity was a

215 little lower, calculated at 90%. Among patients without SSc, anti-Th/To were most often
216 detected in patients with isolated Raynaud's phenomena andILD, and exceptionally with
217 systemic lupus erythematosus, polymyositis, rheumatoid arthritis, primary Sjogren's
218 syndrome and primary immunologic thrombocytopenia.

219

220 Autoantibodies related to SSc are known to be mutually exclusive [25]. In most of the
221 reported studies [19,23,24,26,27], anti-Th/To were not associated with other classical SSc
222 antibodies and cases of co-positivity appear very rare [22].

223

224 Anti-Th/To have an excellent specificity for SSc and their research appears useful, given their
225 association with ILD. However, their frequency is low (3% of SSc patients), in particular in
226 Europe (1% of the patients) and indeed does not sustain their systematic screening in the
227 routine immunological assessment. Mahler et al [20] suggested a higher relevance of anti-
228 Th/To in SSc patients negative for ACA, anti-topo1, anti-PM-Scl, anti-RNP, anti-RNAPolIII,
229 anti-fibrillarine, and anti-NOR 90. Thus, screening of Th/To seems more useful after a
230 negative search for the most common known antibodies.

231

232 Interestingly, in our little series of anti-Th/To positive SSc patients, a speckled nucleolar
233 immunofluorescence pattern on HEp-2 cells was observed. Nucleolar pattern of Th/To
234 antibodies was also described in others studies [15,16,18–20,28]. This pattern was reported as
235 homogenous [29] or speckled [16], as in our cases. Considering these data, a screening
236 strategy based on the nuclear immunofluorescence pattern could increase their sensitivity and
237 could be more cost-effective than a multiantigen Immunodot.

238

239 **Conclusion**

240

241 Based on an exhaustive literature review, anti-Th/To appear as relevant markers of SSc, more
242 frequently observed in patients with a limited cutaneous form of the disease and with an
243 interstitial lung involvement. Their accuracy for SSc diagnosis is of great interest for patients
244 with a suspected SSc disease and with negative SSc-specific antibodies, particularly in case of
245 a speckled nucleolar immunofluorescence pattern observed at the screening of ANAs.

246

247

248

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250 The authors thank Eurobio-Ingen, Les Ulis, France for supplying Immunodot assays for
251 detecting anti-Th/To autoantibodies

252

253 **Conflict of interest**

254 The authors declare non conflict of interest.

255

256

257 **Legend of the Figure**

258

259 **Figure 1: Anti-nuclear antibody pattern observed on HEp-2 cells by indirect**
260 **immunofluorescence (400 x magnification):** anti-Th/To autoantibodies are associated with a
261 speckled-nucleolar pattern that displays a homogeneous staining of nucleoli associated with
262 numerous fluorescent speckles of variable size distributed throughout the nucleoplasm.
263 Metaphase chromosomal plates remain unstained.

264

265 **Legend of the tables**

266

267 **Table 1: Clinical and biological characteristics of patients positive for anti-Th/To**
 268 **antibodies**

269

	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5	Patient 6
Age (years)	57	53	67	60	62	57
Sex	Female	Female	Female	Female	Male	Female
Associated diagnosis	Sjogren's syndrome	Primary biliary cirrhosis	No	No	No	No
Age at diagnosis	55	30	64	45	58	40
ACR/EULAR Criteria	14	16	11	11	11	15
Raynaud's phenomenon	Yes	Yes	Yes	Yes	Yes	Yes
Sclerodactyly	Yes	Yes	Yes	Yes	Yes	Yes
Calcinosis cutis	No	Yes	No	No	No	No
Digital pitting scar	Yes	Yes	No	No	No	No
Telangiectasias	Yes	Yes	Yes	Yes	Yes	Yes
Cutaneous form	Limited	Limited	Limited	Limited	Limited	Limited
Pericarditis	No	No	Yes	No	Yes	No
Muscle damage	Yes	No	No	No	No	No
Upper digestive signs	Yes	Yes	Yes	No	Yes	Yes
Lower digestive signs	No	No	No	No	No	No
Total LungCapacity (%)	91%	101%	86%	97%	93%	102%
DLCO (%)	48%	24%	64%	74%	39%	60%
ILD on chest CT-scan	Yes	Yes	Yes	NC	Yes	Yes
Left Ventricular Function	Normal	Normal	Normal	Normal	Normal	Normal
PAH	No	No	No	Yes	No	No
Level of ANAs (dilutions titers)	>1/1280	>1/1280	>1/1280	>1/1280	>1/1280	>1/1280
Immunofluorescence pattern	SN	SN	SN	SN	SN	SN
<i>Type of auto-antibodies</i>						
Anti-dsDNA (IU/ml)	No	Yes (90)	No	No	No	No
ACA/anti-RNAPol III/anti-topo I (IU/ml)	No	No	No	No	No	No
Anti-Jo1/PM-Scl (IU/ml)	No	No	No	No	No	No
Anti-U1-RNP (IU/ml)	No	No	No	No	No	No
Anti-SSA/SSB (IU/ml)	>131 / 0	62 / 0	0	30 / 0	0	0
Anti-CCP (IU/ml)	0	0	NC	NC	NC	No
Rheumatoid factor level (IU/ml)	60	0	0	0	0	25
Anti-mitochondrial (IU/ml)	0	800	NC	NC	NC	NC
LA or antiphospholipid (UI/ml)	0	0	NC	0	NC	NC
Cryoglobulinemia	0	Positive mixed	NC	NC	NC	NC

270

271 NC: Not communicated; DLCO: diffusing capacity of the lung for carbon monoxide; PAH:

272 Pulmonary arterial hypertension confirmed with a right heart catheterization; LA: Lupus

273 anticoagulant; Anti-phospholipid antibodies included anti-cardiolipin and anti β_2 -glycoprotein

274 1 antibodies; SN: Speckled nuclear nucleolar.

275 **Table 2: Literature review on the frequency of Th/To antibodies in Systemic Sclerosis**
 276 **(SSc)**

Articles	Location	Number of SSc	Dosage method	Anti-Th/To positivity (N) %
Kipnis1990 [28]	USA	84	DOT	(11) 13%
Okano1990 [16]	USA	371	RadioIP	(15) 4%
Falkner 2000 [29]	USA	292	DOT	(28) 9.6%
Jacobsen 2001 [17]	Denmark	174	DOT	(4) 2.3%
Yamane 2001 [18]	Japan	70	RadioIP	(7) 10%
Kuwana 2002 [22]	Japan	303	RadioIP	(14) 4%
Van Eenennaam H 2002 [30]	Netherlands	14	RadioIP	(2) 14.3%
Steen 2005 [31]	USA	1432	DOT	(72) 4.9%
Meyer 2007 [32]	France	127	DOT	(1) 1%
Meyer 2007 [32]	USA	247	DOT	(10) 4%
Hamagushi 2008 [33]	Japan	203	DOT	(7) 3%
Ceribelli 2010 [19]	Italy	261	RadioIP	(8) 3%
Mierau 2011 [34]	Germany	863	DOT	(2) 0.2%
Rodriguez-Reyna 2011 [35]	Mexico	139	DOT	(0) 0%
Van Praet 2011 [24]	Belgium	145	DOT	(3) 2.1%
Villalta 2012 [23]	Italia	210	DOT	(7) 3.3%
Graf 2012 [36]	Australia	129	DOT	(8) 6%
Poormoghim 2013 [37]	Iran	100	DOT	(4) 4%
Mehra 2013 [38]	Canada	528	DOT	(15) 2.8 %
Wielosz 2014 [39]	Poland	97	DOT	(3) 3.4%
Hudson 2014 [40]	Canada	874	DOT	(20) 2%
Malher 2014 [20]	Canada	873	RadioIP	(19) 2%
Chang 2015 [41]	New Zealand	60	DOT	(1).7%
Nandiwada 2016 [42]	USA	1000	DOT	(39) 3.9%

Liaskos 2017 [43]	Greece	131	DOT	0
TOTAL		8727		(300) 3.4%

277 DOT: Multi antigen Immunodot test of anti-Th/To antibodies

278 RadioIP: Radioimmunoprecipitation

279

280

281

282

283 **Table 3: Frequency of anti-Th/To antibodies in Systemic Sclerosis (SSc) patients by**
284 **continent**

Continent	Number of studies	Number of SSc patients	Th/To positivity (N) %
North America	9	5701	(229) 4.0%
South America	1	139	(0) 0%
Europe	9	2022	(30) 1.5%
Asia	4	676	(32) 4.7%
Oceania	2	189	(9) 4.7%

285

286

287

288 **Table 4: Literature review on the frequency of pulmonary fibrosis among Systemic**
 289 **Sclerosis (SSc) patients with anti-Th/To antibodies**

290

Articles	Location	Definition of pulmonary fibrosis in the article	Number of pulmonary involvement among anti-Th/To positive patients (%)
Okano 1990 [16]	USA	CT-scan or PFT	6/14 (42%)
Falkner [29]	USA	CR or CT-scan	6/23 (26%)
Kuwana 2002 [22]	Japan	CT-scan	5/14 (35%)
Mitri 2003 [15]	USA	CR or PFT	46/96 (48%)
Steen 2005 [31]	USA	CR or PFT	45/72 (63%)
Hamagushi 2008 [33]	Japan	CT-scan or PFT	2/7 (29%)
Ceribelli 2010 [19]	Italy	CR or CT-scan	3/8 (38%)
Graf 2012 [36]	Australia	CT-scan or PFT	2/8 (25%)
Malher 2014 [20]	Canada	CT-scan or PFT	10/19 (53%)
Muller 2019	France	CT-scan or PFT	5/6 (83%)
TOTAL			125/264 (47.3%)

291

292 CT-scan = Computerized Tomography scanner

293 CR = Chest Radiography

294 PFT = Pulmonary Function Testing

295

296

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298

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